THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 127 OCTOBER 1, 1939 No. 3

ADRENALIN LACTACIDEMIA: PROPORTIONALITY WITH DOSE

FRED R. GRIFFITH, JR., JULIA E. LOCKWOOD AND F. E. EMERY

From the Department of Physiology, University of Buffalo

Received for publication June 6, 1939

This report is companion to one published recently (Griffith, Lockwood and Emery, 1939) which described the relationship between blood sugar level and rate of intravenous injection of adrenalin and will deal with the blood lactic acid concentration of the same animals following the same periods of adrenalin injection.

PROCEDURE. This is described in detail in the report referred to above; only the more significant points need be repeated here.

Preparation of the animals. Cats under chloralose anesthesia were used exclusively. Preparation consisted merely of insertion of necessary cannulae: tracheal; both carotids—one for blood sampling and the other for registration of blood pressure; venous, in a superficial branch of one femoral for injection. Following these simple operative procedures, 15 to 20 minutes were allowed for stabilization before taking the first blood sample.

Blood sampling. Normals; occasionally two normal blood samples, 15 to 20 minutes apart, were taken preceding injection to establish the magnitude of spontaneous change; more often, to conserve blood volume, one normal sample was considered sufficient.

Following injection, samples were taken immediately, and 10 minutes, and 30 minutes after its completion.

Blood analysis. The blood, removed by pipette from the carotid sampling cannula, was used to prepare a Folin-Wu tungstic acid filtrate; with this, lactic acid determination, in duplicate, was made by the method of Friedemann, Cotonio and Shaffer.

Injection. Parke-Davis adrenalin chloride was diluted with neutral (pH 7.3) isotonic NaCl solution so the amount to be injected per minute was contained in 1 cc. Injection into the femoral vein was always for 5

minutes, 1 cc. per minute, by hand from a 5 cc. syringe, with care to maintain uniformity of rate. The rates employed, with the number of experiments (in parentheses) were, milligrams per kilo per minute: 0.00025 (10); 0.00050 (6); 0.00100 (9); 0.00200 (14); 0.00400 (13); 0.00700 (10); 0.01000 (6). In the previous report on adrenaline hyperglycemia reason was given for not employing rates of injection beyond this maximum.

Control injections of neutral, isotonic NaCl (10 experiments) were similar in amount, rate and duration.

RESULTS. *Normal blood lactic acid level: Intra-individual stability.* Reference has been made to the procedure of occasionally taking two normal blood samples, 15 to 20 minutes apart, preceding injection; this was done in 36 of the total 78 experiments with the average result that the first and second blood lactic acid values were 10.8 and 9.3 mgm. per cent respectively. This seemed to indicate that under the conditions of these experiments the resting normal blood lactic acid level was sufficiently stable to eliminate danger of spontaneous change interfering with interpretation of the result following injection. In the remaining experiments, therefore, one determination of the resting value was considered sufficient.

Inter-individual variability. In spite of this steadiness of resting values in individual animals, inter-individual variation was large, although all animals were treated as uniformly as possible. The statistics of this variability for the 78 animals of this study are: range, 4 to 22; mode, 10; and mean, 11 mgm. per cent; standard deviation, 3.97; coefficient of variation (standard deviation/mean $\times$ 100), 36.1.

This is very similar to the large inter-individual variability of resting, normal blood sugar values for these same animals, described in the preceding report already referred to, with a range from 75 to 265 mgm. per cent and a coefficient of variation of 25.5; i.e., the variabilities of normal, resting blood sugar and of lactic acid are of similar magnitude, the latter being slightly greater as measured by the coefficient of variation. Variations of these two blood constituents are, however, almost completely unrelated, the coefficient of correlation between them being only $+0.100 \pm 0.076$.

In view of this large variability of resting blood lactic acid values it might be expected that the effect of adrenalin would depend not so much or at least not only on rate of injection but in part, at least, on whether blood lactic acid was high or low to begin with. Examination of the data within any one injection group does not substantiate this; and its further elimination as a possible disturbance for the average results reported here would seem to follow from the fact that the average normal blood lactic acid values for the various groups were quite alike. Thus for the groups receiving adrenalin at the various employed rates (in parenthesis) the average resting values in milligrams per cent, were: 11.9 (0.00025), 12.9 (0.00050), 8.0 (0.00100), 10.1 (0.0020), 10.4 (0.00400), 10.0 (.00700) and

9.9 (0.01000). These are sufficiently alike to exclude initial blood lactic acid value as a determining factor in the average result of any given rate of injection.

The effect of control injection of isotonic NaCl. Averages of the 10 experiments in which isotonic NaCl was injected in amount similar to that serving as vehicle for adrenalin in the other experiments showed blood lactic acid values 97, 93 and 100 per cent of normal at the end of the 5-

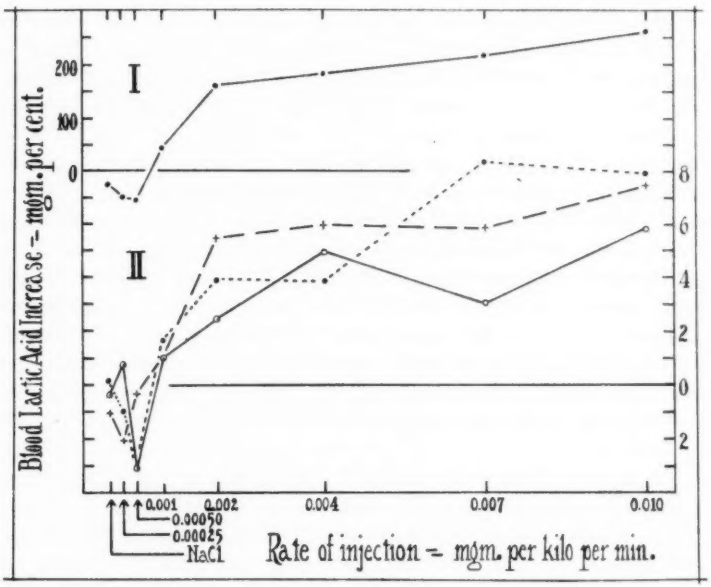


Fig. 1. The effect upon blood lactic acid concentration of intravenous injection of isotonic NaCl solution and of adrenalin at rates of 0.00025, 0.00050, 0.00100, 0.00200, 0.00400, 0.00700 and 0.01000 mgm. per kilo per minute for 5 minutes.

II. The lower group of 3 curves: continuous line, values immediately upon completion of the 5-minute injection; dashed line, values 10 minutes after the end of injection; dotted line, values 30 minutes after the end of injection.

I. Upper single curve: total change during the 35-minute period including the 5-minute injection and the 30 minutes following.

minute injection, and 10 minutes, and 30 minutes after its completion. The constancy of the blood lactic acid level over this 35-minute experimental period would seem to be the significant conclusion from this series of control experiments. Injection of 5 cc. of isotonic NaCl, withdrawal of blood samples, nor mere lapse of time and spontaneous change would seem to introduce any disturbance capable of masking or modifying a specific adrenalin effect. This corresponds with previous experience of Cori, Cori and Buchwald (1930).

These control values have been used as the initial points for the curves of figure 1.

The effect of adrenalin injection. Figure 1 presents the results in two ways: part II includes three curves defining blood lactic acid immediately (continuous line), 10 minutes (dashed line), and 30 minutes (dotted line) after injection was completed; the single curve of part I depicts the approximate total change effected by each rate of injection for the entire 35-minute experimental period, as obtained by summing the products of lactic acid values at end of injection, and 10, and 30 minutes after, when multiplied by 5, 10 and 20 (minutes), respectively. Though not an exact summation of the area beneath the individual blood lactic acid curves for each rate of injection it provides a figure easily calculated and sufficiently accurate for the present purpose.

Threshold. The lowest rate of injection consistently increasing blood lactic acid concentration is 0.001 mgm. per kilo per minute. The total effect of the two rates of injection below this (0.00025 and 0.00050 mgm. per kilo per minute) appears to be a decrease (fig. 1, I); but this is only slightly greater than the effect of control injection of NaCl alone and is probably merely projection into the injection and recovery periods of the spontaneous normal decline described in a previous section and characteristic of these animals under these experimental conditions. At all events this slight decline is probably in no way related to the lowering of blood lactic acid which adrenalin is reported to cause in fatigued rabbits (Inawashiro, 1933).

A threshold of 0.001 mgm. per kilo per minute is approximately four times that reported by Goldblatt (1933) for cats under amytal or urethane, and 10 to 20 times greater than found by Cori, Cori and Buchwald for unanesthetized rabbits. Goldblatt, however, employed infusions of 60-minutes' duration and the single protocol given indicates very little change in blood lactic acid within the much shorter period covered by our experiments. The same is true in part of the experiments of the Coris; it would seem doubtful from their curves whether they would have attached importance to their lowest rate of injection had they stopped at the end of 5 minutes instead of continuing for 2 hours; their 0.0001 mgm. per kilo per minute probably would have given positive results even with the shorter period of injection and the difference between this and the 10-fold higher threshold found in this work may be taken as a measure of elevation of threshold due to chloralose anesthesia.

Reference to our previous report will show that this threshold for lactic acid is twice the rate of injection (0.00050 mgm. per kilo per minute) required to initiate elevation of blood sugar in these same animals.

Proportionality between rate of injection and blood lactic acid elevation. Consideration of this relationship will be facilitated by dealing separately

with the values attained 1, at the end of the 5-minute injection period; 2, 10 minutes, and 3, 30 minutes after injection; and 4, the total summated effect for the entire 35-minute experimental period.

1. *Blood lactic acid at the end of the 5-minute injection period* (fig. 1, II, continuous line): starting with the effect of injection at the rate of 0.001 mgm. per kilo per minute (+12 per cent) it will be seen blood lactic acid elevation rises uniformly (to +47 per cent) as injection is increased to four times this rate. In view of all the facts it would seem probable that the decline (to +30 per cent) at 0.007 mgm. per kilo per minute is aberrant since 0.01 again effects an elevation (+59 per cent) even greater than 0.004 mgm. per kilo per minute. Very probably, therefore, the true course of events is a sharp rise as rate of injection is increased to 0.004 and thereafter a slightly rising plateau as rate of injection is increased to 0.01 mgm. per kilo per minute.

2. *Blood lactic acid 10 minutes after injection* (fig. 1, II, dashed line): at the threshold rate of injection (0.001 mgm. per kilo per minute) blood lactic acid does not rise or fall during the ten minutes after injection. Thereafter, however, at all higher rates of injection, it continues to increase during this interval and is uniformly higher 10 minutes after injection than at the moment injection was stopped. A similar condition was noted in our previous description of adrenalin hyperglycemia, blood sugar following almost all rates of injection continuing to rise after injection stopped; the explanation there advanced was neither certain nor perspicuous and need not be repeated. But since such an effect would seem at variance with the accepted idea of the transitoriness of adrenalin action and since it is seen in connection with both of these blood constituents, it would seem deserving of serious consideration.

3. *Blood lactic acid 30 minutes after injection* (fig. 1, II, dotted line): again, at threshold rate of injection (0.001 mgm. per kilo per minute) blood lactic acid changes very little during this 20-minute interval since the previous blood sampling. After injection at 0.002 and 0.004 mgm. per kilo per minute blood lactic acid has declined during this interval from the maximum reached 10 minutes after injection (+53 and +57 per cent, respectively), but has not returned to normal (being still +39 and +37 per cent, respectively). Except that a more rapid return to normal might have been anticipated this decline seems metabolically reasonable. On the other hand, altogether unexpected is the upward trend of blood lactic acid level during this late interval after previous injection of 0.007 and 0.010 mgm. per kilo per minute. After injection at these rates, blood lactic acid shows no indication of returning to normal during the 30-minute recovery period covered by this work. Thus, immediately, 10, and 30 minutes after injecting 0.007 mgm. per kilo per minute, blood lactic acid increases are +30, +58 and +83 per cent; the corresponding increases

after 0.01 mgm. per kilo per minute are +59, +75 and +80 per cent. Though the greatest rate of increase occurs during injection itself, these higher rates of injection seem to impart a momentum to lactic acid mobilization which is not completely exhausted during a subsequent half-hour period. This in all probability is only an intensification of the lesser effect observed at lower rates of injection and noted in the previous section and which resulted in a continuing increase to maximum values 10 minutes after injection stopped.

This effect is quite different from that observed with blood sugar as described in our previous report; maximum blood sugar values occurred during, or 10 minutes after injection and recovery toward normal was not only always appreciable during the 20-minute interval under consideration here, but proceeded more rapidly after the two highest rates of injection than after the lesser ones. This suggestion of a difference in the underlying mechanisms whereby blood sugar is mobilized on the one hand and blood lactic acid is increased on the other is in line with the previously mentioned lack of correlation between the resting normal variations of these two blood constituents; and is further indicated by the fact, previously described, that adrenalin hyperglycemia is definitely maximum following injection at the rate of 0.004 mgm. per kilo per minute whereas the total lactic acid increase is directly proportional to dosage as will be evident in the following.

4. *Total, summated lactic acid increase for the entire 35-minute experimental period* (fig. 1, I): this curve is self-evident and is clearly merely the expression in toto of the evidence implicit in each of the three curves previously considered.

COMMENT. The most significant feature of these results is their marked dissimilarity to the hyperglycemia occuring in these same animals in response to these same injections (previous report). Although lactic acid formation and release of blood sugar might be regarded as results of direct cellular action, proportional to the concentration of circulating adrenalin up to that established by injection at the rate of 0.004 mgm. per kilo per minute, beyond this similarity stops: lactic acid continuing to increase in proportion to rate of adrenalin injection; hyperglycemia diminishing as rate of injection is further increased.

Complications ensuing from adrenalin vascular effects suggest themselves as possibly contributory to this differential action. At least during injection, constriction proportional to dosage might ultimately bar access of injected adrenalin to glucogenic sites and thus diminish hyperglycemia, with simultaneous increase of lactic acid formation by anoxic glycogenolysis. Perhaps, also, in this connection too little significance has been assigned to the apparent decrease of blood lactic acid concentration resulting from the two lowest rates of injection. Although sufficiently quantitative measurements of blood pressure are not available, the usual

effect during these injections was slight depression; the same dose (0.001 mgm. per kilo per minute) that was threshold for blood lactic acid elevation being also the lowest that was consistently pressor. Vasodilatation with only slight decrease of general blood pressure might conceivably result in localized increased flow and oxygen supply and diminished escape of lactic acid into the blood.

At present, however, such speculation, though unavoidable, is perhaps neither wise nor profitable; and aside from any value these results may ultimately have in explaining the mechanism of adrenalin action, they have the immediate and practical consequence of emphasizing the great importance of the control of the rate of administration in any quantitative study of the metabolic effect of this hormone.

SUMMARY

Intravenous injection of adrenalin at rates of 0.00025, 0.00050, 0.00100, 0.00200, 0.00400, 0.00700 and 0.01000 mgm. per kilo per minute for five minutes affects the blood lactic acid level of chloralose anesthetized cats as follows:

1. The approximate minimal effective dose for blood lactic acid elevation is 0.00100 mgm. per kilo per minute.

2. Maximum blood lactic acid values are never attained during the injection period:

a. With rates of injection of 0.002 and 0.004 mgm. per kilo per minute, maximum is attained 10 minutes after injection and is followed by return toward but not to normal during the subsequent 20-minute interval.

b. With rates of injection of 0.007 and 0.010 mgm. per kilo per minute blood lactic acid continues to increase throughout the entire half-hour following injection.

3. Total blood lactic acid increase for the entire 35-minute experimental period is rapid up to rate of injection of 0.002 mgm. per kilo per minute and thereafter increases less rapidly but steadily in direct proportion to dosage.

4. There is a slight indication that the two lowest rates of injection, which were also usually depressor, effect a decrease in blood lactic acid concentration. This suggestion of the possible significance of its concomitant vascular effects may also find application in explanation of the progressive increase of blood lactic acid and decrease of hyperglycemia (previous report) resulting from injection at the two highest rates that were used.

REFERENCES

CORI, C. F., G. T. CORI AND K. W. BUCHWALD. This Journal **93**: 273, 1930.
GOLDBLATT, M. W. J. Physiol. **78**: 96, 1933.
GRIFFITH, F. R., JR., J. E. LOCKWOOD AND F. E. EMERY. This Journal **126**: 299, 1939.
INAWASHIRO, T. Tohoku J. Exper. Med. **20**: 553, 1933.

THE RÔLE OF SEX, ESTROGENIC HORMONE, FASTING AND DIURESIS IN THE RESPONSE OF CROP-SACS OF PIGEONS TO PROLACTIN

ROBERT W. BATES, OSCAR RIDDLE AND ERNEST L. LAHR

From the Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Received for publication June 24, 1939

Folley and White (1) observed that the weight increase in crop-sacs of pigeons following injections (subcutaneous, for 6 days) of prolactin is partly inhibited in both sexes by simultaneous injections of estradiol monobenzoate in sesame oil. Very recently, from a more extensive study of the inhibiting effect of estrone, Folley (2) reports a maximum inhibition of 50 per cent. These decreases of expected growth in the crop-sacs were accompanied also by some decrease in the body weights of the treated birds. Folley further reported that the rate of absorption of estrone from the site of injection (volume of oil used) partly determines the extent of inhibition of the response to prolactin; sex was thought to play no part in this inhibition. Upon repeating the Folley and White experiments we find that dihydroestrone notably inhibits the crop-sac response to prolactin; that equal amounts of *subcutaneously* injected prolactin (4 days), with or without estrogenic hormone treatment, usually produces heavier crop-sacs in male Carneau pigeons than in females; and that this sex difference is nearly or quite absent when the intramuscular route of injection (4 days) is used.

We have studied also the mechanism through which the expected action of prolactin on crop-sac growth is diminished by estrogenic hormone. The effect of diuresis (theobromine) alone and of fasting alone on the response of the crop-sac each proved to be quite large, and the estrogen-treated pigeons were found to show a significant increase in water intake and a diminished food intake. The decrease in food intake and the coincident increase in water (and prolactin) elimination which result from the administration of large dosage with estrogen clearly account for much, though probably not all, of its ability to inhibit the crop-sac response to prolactin.

MATERIAL AND METHODS. White Carneau squabs at 6 weeks after hatching were used throughout. Prolactin injections (in 0.4 cc., except as indicated) were made once daily for 4 days with autopsy 96 hours after

the first injection so that crop-sac weights could be converted into units. For comparative purposes the crop-sac weights are all converted to "units" with the aid of our standard table (3; p. 1102); the "units" used here, however, are not true Riddle-Bates units because they are not corrected for the racial factor (for Carneau pigeons division by 1.2 is required), and because those "units" derived from subcutaneously injected birds are not corrected for the differences resulting from use of that route of injection. Subcutaneous injections were made over the breast muscle; intramuscular injections were made into that muscle. Except in the fasting experiments food and water in excess were offered (and intake measured) to the squabs in groups, not individually.

TABLE 1

The rôle of sex in the response of crop-sacs to prolactin injected intramuscularly

PROLACTIN SAMPLE	TOTAL DOSE	SEX	NUMBER OF BIRDS	BODY WEIGHT	WEIGHT OF CROP-SACS	σ_{mean}	UNITS	SEX QUOTIENT ♂/♀
	mgm.			*grams*	*mgm.*			
LP5	1.6	♂	5	523	2,750	±315	14.7	1.31
		♀	5	480	2,490	±246	11.2	
LP7	3.2	♂	6	470	3,190	±265	23.0	1.26
		♀	4	444	2,960	±254	18.2	
469	4.0	♂	13	456	2,455	±181	10.7	1.29
		♀	12	427	2,217	±211	8.3	
AW*	1.0	♂	4	492	2,028	±285	7.0	0.84
		♀	4	432	2,207	±375	8.3	
657	2.0	♂	32	477	3,520	±151	32.0	1.33
		♀	18	459	3,250	±206	24.0	

* A sample of "crystalline" prolactin kindly supplied by Dr. Abraham White

RESULTS. *A sex difference in response of crop-sacs to prolactin.* In the type of pigeons used by us the response to prolactin injected intramuscularly for 4 days shows no significant sex difference (table 1). Four of the 5 sex quotients are greater than 1.0, but the observed differences are statistically far from significant. A correction of crop-sac weights for sex difference in body weight would bring these sex quotients (with the exception of sample AW) still closer to a value of 1.0. In some experiments with preparation no. 469 reported earlier (4) the sex quotient from intracutaneous injection was 1.25, while the quotients from subcutaneous injection at various dosage levels were 0.9, 1.45, 1.6, and 1.8, none of which were statistically significant.

Table 2 shows that a statistically significant sex difference in crop-sac

response often occurs following injections of prolactin by the subcutaneous route. This was observed in the single group studied by Folley and White, but apparently not in the more complete study by Folley (in which, however, the age of the birds used was not accurately known). In groups 4, 5, 6 and 7 of table 2 (i.e., groups having acceptable numbers of both sexes) there is a significant difference of at least 3 times the standard error of the means, and in only one such case (group 2) is a smaller sex difference found.

TABLE 2

Effects of fasting and of simultaneous dosage with dihydroestrone upon the response of male and female crop-sacs to prolactin

All injections made subcutaneously

GROUP	SIMULTANEOUS TREATMENT	SEX	NUMBER OF BIRDS	WEIGHT OF CROP-SACS	σ_{mean}	PROLACTIN UNITS	SEX QUOTIENT ♂/♀	REDUCTION (IN UNITS) BY SIMULTANEOUS TREATMENT
	Prolactin no. 437—5 mgm./day							
				mgm.				*per cent*
2	Sesame oil	♂♂	9	5,120	±441	166	1.55	0
		♀♀	6	4,690	±385	107		0
3	None (437 = 0.05 cc.)	♂♂	8	5,100	±150	162	6.0	0
		♀♀	2	3,320	±720	26		0
4	Fasted	♂♂	4	4,456	±160	83	3.4	49*
		♀♀	6	3,580	±170	34		54*
5	10 mgm. dihydroestrone, twice†	♂♂	9	3,233	±202	24	3.0	85
		♀♀	6	2,173	±250	8		89
6	10 mgm. dihydroestrone, once‡	♂♂	5	3,462	±130	30.5	2.4	82
		♀♀	5	2,596	±130	12.5		83
	Unfractionated anterior pituitary extract no. 582—10 mgm./day							
7	Sesame oil	♂♂	5	5,502	±130	244	4.0	0
		♀♀	5	4,144	±220	61		0
8	10 mgm. dihydroestrone, twice†	♂♂	8	4,080	±193	57	2.4	75
		♀♀	2	3,240	±480	24		61

* Calculated on basis of 164 units (for 17 males, = 100 per cent) and 75 units (for 8 females, = 100 per cent) shown by control groups 2 and 3.

† Injected 9 and 4 days before autopsy.

‡ Injected 4 days before autopsy.

Also, in two tests (groups 3, 8) in which females were very inadequately represented the male crop-sac weights were definitely larger. It will be observed that this greater response of male crop-sacs usually attends the injection of prolactin by the subcutaneous route, and its occurrence is independent of simultaneous injections of estrogen.

We can offer no explanation for this exhibition of a sex difference following one route of injection and not of another. Since the pigeons were studied at six weeks after hatching, and when quite immature sexually, it is improbable that their own sex hormones measurably affected the results obtained in our tests.

For assay purposes and quantitative tests (using 4 daily injections) the intramuscular route of injection now seems definitely preferable to the subcutaneous route because the former avoids the complication of a sex difference in the response (the sex of squabs is learned only by biopsy). In an earlier study (4) it was shown that the crop-sacs from groups of birds injected subcutaneously were larger than those given intramuscular injections of prolactin. The results recorded here indicate that the greater part of the difference earlier attributed to route of injection was in fact contributed by the males used in those tests.

Effect of dihydroestrone on crop-sac response to prolactin. When injected simultaneously with prolactin large doses of dihydroestrone greatly diminish the increase in crop-sac weight which would result from the injection of prolactin alone (table 2). This estrogen was dissolved in sesame oil and injected subcutaneously into three groups of birds. Group 6 received only one injection of dihydroestrone; groups 5 and 8 received two injections (according to the procedure of Folley and White), the first being given 5 days before the initial prolactin injection and the second at the beginning of the series of prolactin injections. Our tests indicate only moderate effects of the first or preliminary injection of dihydroestrone on the crop-sac response, with some accentuation of effects on food and water consumption and on body weight (table 3, groups 5 and 8 compared with group 6). When used with prolactin no. 437 the dihydroestrone dosage reduced the crop-sac response (in terms of "units") by 82 to 89 per cent, and when used with the unfractionated preparation no. 582 it reduced this response by 61 to 75 per cent (table 2). These reductions are highly significant for the male (or larger) groups in the case of both pituitary fractions (nos. 437, 582).

Effect of fasting on crop-sac response to prolactin. Since the food intake was notably reduced by the dihydroestrone treatment (table 3) it was of interest to determine in one group of birds the effect of complete fasting during the 4-day period of prolactin treatment. It will be seen (group 4, table 2) that the effectiveness of the prolactin, in terms of units, was reduced by 49 to 54 per cent merely by withholding all food.

Effect of diuresis on crop-sac response to prolactin. Ability of the kidney to excrete prolactin is well established. The probability that dihydroestrone causes a more rapid elimination of prolactin by the kidneys was suggested by the high daily water intake of birds thus treated (table 3).

TABLE 3

Showing changes in body weight, organ weight and food and water intake in variously treated groups of young Carneau pigeons

GROUP	SIMULTANEOUS TREATMENT	NUMBER OF BIRDS	BODY WEIGHT ± CHANGE*	FOOD	WATER	OVIDUCT	TESTES
	No prolactin						
			grams	*grams/bird/day*	*cc./bird/day*	*mgm.*	*mgm.*
1	Dihydroestrone 10 mgm., twice	10	462 + 3	30† 33*	72† 126*	5,839	55
	Prolactin no. 437—5 mgm. per day*						
2	Sesame oil	15	492 + 21	41	75	38	69
3	None (437 = 0.05 cc.)	10	450 + 50				77
4	Fasted	10	440 − 35	0	55	26	37
5	Dihydroestrone 10 mgm., twice	15	475 − 40	22	106	5,973	48
6	Dihydroestrone 10 mgm., once	10	425 + 5	29	88	1,912	63
	Unfractionated anterior pituitary extract no. 582—10 mgm. per day*						
7	Sesame oil	10	462 + 14	31	64	76	264
8	Dihydroestrone 10 mgm., twice	10	465 − 10	31	112	6,775	316
	Prolactin no. 657—0.5 mgm. per day*						
9	None (subcutaneous)‡	10	442 + 60	41	75		35
10	Theobromine (subcutaneous)	9	431 + 8	22	111		23
11	None (intramuscular)‡	10	449 + 46	39	87		39
12	Theobromine (intramuscular)	10	494 − 45	12	140		28

* During last four days.
† During first five days.
‡ Route of prolactin injections.

The effect of diuresis on the crop-sac response, as this occurs under theobromine dosage (75 or 100 mgm. of theobromine, twice daily) was therefore measured (table 4). The level of dosage used led to an increase in water intake comparable with that produced in our experiments by dihydroestrone, and it also reduced the food intake to a similar or greater

degree (see table 3). The tests with theobromine thus show the effect on the crop-sac response (prolactin inhibition) of this degree of reduction of food intake and of increased excretion by the kidney. Under theobromine the crop-sac response was reduced by 62 per cent—an amount which was the same for both routes of prolactin injection but which seems significantly less than the reductions observed under heavy dosage with dihydroestrone.

DISCUSSION. The specific or primary action of dihydroestrone is of course a stimulation of the secondary sex apparatus. In our tests the weights of oviducts were increased roughly 100 times in 9 days (groups 1, 5 and 8, table 3) and over 30 times (group 6) in only 4 days by the high dosage used. It must be noted, however, that dihydroestrone is now

TABLE 4

Quantitative effect of diuresis upon crop-sac response to prolactin

All birds injected daily with 0.5 mgm. prolactin no. 657

GROUP	SIMULTANEOUS TREATMENT (BY MOUTH)	ROUTE OF PROLACTIN INJECTION	NUMBER OF BIRDS	WEIGHT OF CROP-SACS	σ_{mean}	PROLACTIN UNITS	REDUCTION BY SIMULTANEOUS TREATMENT
				mgm.			*per cent*
9	None	Subcutaneous	10	3,293	±346	25.5	
10	Theobromine, 75 mgm. twice daily	Subcutaneous	9	2,343	±261	9.6	62
11	None	Intramuscular	20	3,232	±160	23.8	
12	Theobromine, 100 mgm. twice daily	Intramuscular	10	2,298	±352	9.2	61

known to exert in pigeons still other important physiological actions on the blood calcium, the blood fat and on diuresis.

The diuretic action of dihydroestrone was greater during the last 4 days than during the first 5 days—as is shown in group 1 (table 3) where both values are given and where the estrogen only was injected. Prolactin has been observed regularly in this laboratory to cause an increase in body weight when given in the quantity used in these experiments to either normal or hypophysectomized pigeons; and these increases in body weight have been found associated with an increase in food intake. Dihydroestrone given alone did not cause actual loss in body weight in controls (group 1, table 3) treated over the full 9-day period, nor in groups 5 and 8 during the preliminary 5 days of dihydroestrone treatment; but, immediately upon the initiation of prolactin dosage the birds in groups 5 and 8 began to lose weight. The loss in weight observed in groups 5 and 8 was limited entirely to the period of 4 days of prolactin treatment. When

pigeons were simultaneously treated with dihydroestrone we thus find that prolactin—and also an unfractionated pituitary extract (no. 582) which surely contained what others call "growth hormone"—resulted in a loss instead of a gain in body weight. In short, estrogen inhibits both the crop-sac response and the bodily growth response in pigeons.

Blood calcium is greatly increased in pigeons by estrogenic hormones (5, 6). At the end of treatment the 10 pigeons of group 8 had an average serum calcium value of 47.1 mgm. per 100 cc. in contrast to their normal value of 10.4 mgm. A marked action of estrogens on the blood fat of fowl has been recently noted (7, 8), and this has been found also in pigeons (Riddle and Senum, unpublished). A "sodium retaining effect" of sex hormones, of dihydroestrone especially, has been reported (9, 10), and an effect of estrogens on the anterior pituitary is well recognized. Again, the reduction in crop-sac response obtained here with dihydroestrone is of the same magnitude (seven-eighths) as that found by this laboratory to result from hypophysectomy (11). Obviously some of the above noted effects of dihydroestrone may be indirect results of pituitary stimulation or inhibition and conceivably capable of modifying the fate or effectiveness of injected prolactin. Finally, one should here recall the complicating and unexplained fact that the crop-sacs of adult pigeons (which produce moderate or physiologically normal amounts of sex hormones) are much more sensitive to prolactin than are those of young pigeons in which practically no sex hormone is produced (12). It seems probable, however, that the inhibiting action of estrogens observed in the present study results from the use of abnormally high levels of dosage of the estrogen.

The results described here indicate that high dosage with dihydroestrone has an effect on the crop-sac response in the pigeon which can be accounted for largely but probably not wholly on the basis of its action on food intake and water excretion. A further or residual effect of this estrogen may involve a local direct antagonism in the crop-sac tissue, but it is more probable that merely one or another of the general systemic effects of high dosage of dihydroestrone on metabolism is involved in this diminished response of crop-sac tissue.

SUMMARY

When prolactin is injected subcutaneously into immature pigeons the crop-sacs of males usually give a greater (growth) response, but following intramuscular injections of prolactin there is little or no sex difference in response.

Confirming Folley and White large doses of dihydroestrone markedly inhibit the action of prolactin on the response of the crop-sacs of both sexes.

In terms of units of prolactin complete fasting during the 4-day period

of injection reduced the crop-sac response to 50 per cent. Theobromine-induced diuresis, accompanied by a 30 to 50 per cent voluntary reduction in food intake, reduced this response to 40 per cent. Massive doses of dihydroestrone, accompanied by both diuresis and diminished food intake, reduced the response to 15 to 25 per cent of that of normal untreated pigeons.

The apparently antagonistic action of estrogen against prolactin is found thus to be largely though probably not wholly the result of the diuresis and reduced food intake which dihydroestrone induces in pigeons; any additional or residual effect is thought likewise to rest not upon a local and specific antagonism but upon one or another of the general effects of dihydroestrone on metabolism.

Estrogen injected simultaneously with prolactin into pigeons inhibits the gains in body weight which, like the response of growth in the crop-sacs, characterize the normal action of prolactin in pigeons; and this inhibition of gain in body weight is apparently produced in the same indirect ways as is the inhibition of the crop-sac response.

REFERENCES

(1) Folley, S. J. and P. White. Nature **140:** 505, 1937.
(2) Folley, S. J. Endocrinology **24:** 814, 1939.
(3) Riddle, O. and R. W. Bates. Sex and internal secretions. Chap. 20, Baltimore, 1939.
(4) Bates, R. W. and O. Riddle. Proc. Soc. Exper. Biol. and Med. **34:** 847, 1936.
(5) Riddle, O. and L. B. Dotti. Science **84:** 557, 1936.
(6) Pfeiffer, C. A. and W. U. Gardner. Endocrinology **24:** 485, 1938.
(7) Lorenz, F. W., I. L. Chaikoff and C. Entenman. J. Biol. Chem. **126:** 763, 1938.
(8) Zondek, B. and L. Marx. Nature **143:** 378, 1939.
(9) Thorn, G. W. and G. A. Harrop. Science **86:** 40, 1937.
(10) Harrop, G. A. Cold Spring Harbor Symposia on Quant. Biol. **5:** 375, 1937.
(11) Schooley, J. P., O. Riddle and R. W. Bates. Proc. Soc. Exper. Biol. and Med. **36:** 408, 1937.
(12) Riddle, O., R. W. Bates and S. W. Dykshorn. This Journal **105:** 191, 1933.

FACTORS AFFECTING THE TOXICITY OF POTASSIUM[1]

ALEXANDER W. WINKLER, HEBBEL E. HOFF AND PAUL K. SMITH

From the Department of Internal Medicine, the Laboratories of Physiology, and of Pharmacology and Toxicology, Yale University School of Medicine, New Haven

Received for publication June 29, 1939

In a previous communication (10) the cause of death following the intravenous injection of potassium salts has been ascribed to diastolic cardiac arrest, occurring only when a rather definite critical concentration of potassium in the serum is attained. Although somewhat variable this critical concentration was usually close to 15 mEq. per liter. The characteristic series of electrocardiographic changes, appearing as the concentration of potassium increased, was entirely reversible until this critical level had been attained. Death was associated with a complete disorganization of the ventricular complex.

The present study is an extension of these observations. Mixtures of calcium and potassium salts were injected simultaneously, to determine whether the excess of calcium would affect either the critical fatal concentration of potassium or the characteristic disorganization of the ventricular complexes. In another series of experiments potassium salts alone were injected, and skeletal muscle twitches at frequent intervals were recorded along with frequent electrocardiograms during the injection. It was thus possible to compare the behavior of skeletal and of cardiac muscles while being perfused simultaneously with the same concentration of potassium.

PROCEDURE. Dogs under morphine analgesia or nembutal anesthesia were employed. A gastrocnemius soleus preparation was isolated with intact blood supply and attached to a myograph for the muscle twitch experiments. Supramaximal stimuli by shielded electrodes applied to the motor nerve were used throughout. The solutions were injected at a slow uniform rate into the femoral vein. In certain animals blood pressures were recorded directly from the femoral artery; the effects on the blood pressure are to be reported elsewhere. Three types of solution were injected: *a*, 0.154 M KCl alone; *b*, 0.154 M mixture of KCl and $CaCl_2$, the K:Ca molar ratio being 8:1; and *c*, 0.154 M mixture of KCl and $CaCl_2$,

[1] Aided by grant no. 354 of the Committee on Therapeutic Research of the American Medical Association, by the Ella Sachs Plotz Fund and by the Fluid Research Fund of the Yale University School of Medicine.

the K:Ca molar ratio being 4:1. Electrocardiograms from lead II were obtained at intervals, and blood samples for analysis were withdrawn from the femoral vein before injection and from the heart at death. Care was taken not to allow the infusion to continue beyond the time of death. Potassium was determined by Hald's modification of the method of Shohl and Bennett (3); and calcium by titration of the oxalate after direct precipitation from the serum. These methods are identical with those previously employed (10).

RESULTS. *Effects of potassium chloride plus calcium chloride.* In figure 1 the concentrations of potassium in the serum at death are compared in two series of experiments. In one series, potassium salts alone were injected, while in the other calcium was injected simultaneously with the potassium. In the control series the concentration of potassium in the serum at death ranged from 12 to 20 mEq. per liter, while in the com-

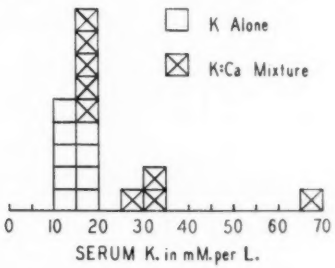


Fig. 1. Concentration of potassium in the serum at death. Each plain square represents one experiment in which potassium alone was given; each cross hatched one in which a potassium calcium mixture was given.

bined injection series four of the nine animals died with concentrations of potassium greatly exceeding this range. The other five fell in the upper range of control values. The two series are sufficiently comparable in all other respects to permit the conclusion that the presence of calcium was the factor responsible for the longer survival. The protocols of the nine combined injection experiments are summarized in table 1, while the data from five of the control experiments are presented in table 2. (The protocols of the other four control experiments are summarized elsewhere (10).) Anesthetics in the control series included both morphine and nembutal, while in the combined series morphia, nembutal and amytal were used. Although one of the high values occurred in the only animal to receive amytal, other high values were found in morphinized animals, while a normal value was observed in one which had received nembutal. No correlation exists between rate of injection and the concentration of potassium in the serum at death, therefore the slightly more rapid rate of

injection in the combined series than in the control series is probably without significance. In every respect the changes in the electrocardiographic

TABLE 1

KCl and $CaCl_2$ injected together

EXPERIMENT NUMBER	RATIO OF MOLS K TO Ca IN SOLUTION INJECTED	WEIGHT OF DOG	AMOUNT	TIME	RATE AS CC. 0.154M, K PER KILO PER MINUTE	K		Ca		CONCENTRATION OF K ASSOCIATED WITH ELECTROCARDIOGRAPHIC CHANGES			
						Initial	Final	Initial	Final	T wave elevation	S-T depression	P wave disappearance	Intraventricular block
		kgm.	*cc.*	*minutes*		*mM per liter*	*mM per liter*	*mgm. per 100 cc.*	*mgm. per 100 cc.*				
2	8:1	14.7	828	103	0.51	3.9	18.5	11.5	33.0	5.0		9.0	17.2
3	8:1	11.6	167	15	0.87	4.1	15.8	10.9	23.8	8.5	8.5	9.2	
4	8:1	7.2	233	32	0.88	3.9	32.5	10.4	34.8	6.2	8.7	15.0	21.5
5	8:1	9.1	79	9	0.89	4.0	18.9	10.2	23.5	7.5	7.5	10.0	12.5
6	8:1	6.0	99	17	0.85	4.1	31.7	11.8	33.6	10.5	10.5	14.0	17.5
8	4:1	9.1	200	15	1.22	6.0	69.4	9.5	64.5	14.0	14.0	35.0	35.0
9	4:1	9.1	132	12	0.97	3.8	27.0		37.9	8.0	8.0	10.5	10.5
10	4:1	13.6	464	24	1.14	5.0	16.7	10.6	26.7	6.0	6.0	7.5	12.5
14	4:1	6.3	202	42	0.60		16.5		24.0	5.8		15.6	15.6

The concentration of each solution was so adjusted that the combined concentration of KCl and $CaCl_2$ was 0.154M. Morphine sulfate was used as an anesthetic in all experiments except number 8, in which sodium amytal was given.

TABLE 2

Muscle twitch series. KCl (0.154M) alone injected

EXPERIMENT NUMBER	WEIGHT	AMOUNT	TIME	RATE AS CC. PER KILO PER MINUTE	K		ELECTROCARDIOGRAPHIC CHANGES				MUSCLE TWITCH TENSION	
					Initial	Final	T wave	S-T depression	P wave disappearance	Intraventricular block	Initial	At moment of cardiac arrest
	kilo	*cc.*	*minutes*		*mM per liter*	*mM per liter*	*mM per liter*	*mM per liter*	*mM per liter*	*mM per liter*	*grams*	*grams*
1	12.7	386	53	0.57	5.0	14.0	6.5		11.2	11.2		
7	25.0	268	21	0.51		13.1	9.0	11.0	12.5	12.5	3,900	3,800
11	9.6	90	13	0.70	4.9	12.5	6.0	6.0	7.5	8.2	1,800	2,000
12	17.0	235	27	0.51		19.5	6.3	7.0	8.0	10.5	500	640
13	14.1	95	9	0.75		19.5	8.5	11.0	11.0	11.0	1,900	2,350

Nembutal used as an anesthetic in all experiments except number 1, in which morphine sulfate was given.

complexes were identical with those associated with the injection of potassium alone. However, in those animals which died at abnormally high levels of serum potassium the preliminary changes, such as disappearance

of P waves and the development of intraventricular block, also first appeared at higher concentrations of potassium than in the control series. There is every evidence that in combined injection experiments the animals actually did die from the effects of the potassium rather than from the increased concentration of calcium, since the level of calcium at death did not exceed 40 mgm. per cent except in one instance. These concentrations are too low to produce death by arrest from calcium alone (4). Just preceding death the ventricular complexes became totally disorganized, a behavior characteristic of potassium (10).

The earliest electrocardiographic change detected following administration of potassium was an increase in the amplitude of the T wave, which seemed to progress in proportion to the increase in serum concentration of potassium until the onset of intraventricular block made it impossible to detect a T wave (fig. 2). Thomson has noticed a similar relationship in

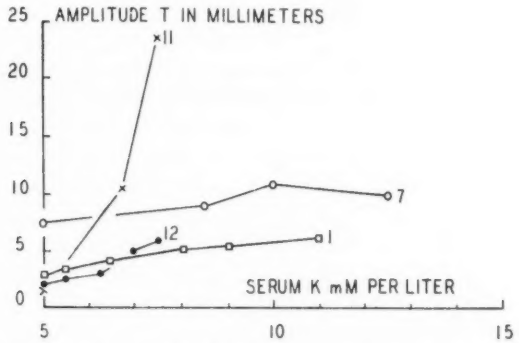


Fig. 2. Relationship between height of T wave and concentration of serum potassium during injection of KCl alone.

man (8). In the combined injection experiments the amplitude of the T wave was proportional to the concentrations of potassium in the serum (fig. 3), only to the point when the P waves disappeared; after this point increase in concentration of serum potassium failed to cause a proportional augmentation in the T wave.

In all five of the twitch experiments summarized in table 2 the skeletal muscle was contracting vigorously at the moment of cardiac arrest. Indeed in three of the four the height of the twitches had actually increased. Thus concentration of serum potassium sufficient to arrest the heart with disorganized ventricular complexes was not in any apparent way toxic to the skeletal muscle.

Discussion. The high concentrations of potassium in the serum at death in the series of combined injections, though not invariably present, occurred too frequently to be a chance phenomenon, and the difference in

the two series is statistically significant. It seems natural to associate it in some way with the antagonism between these ions in their effect upon the amphibian heart. If the heart of a frog is perfused with a solution containing an excess of potassium, it is arrested in diastole; if with an excess of calcium it is arrested in systole (1). A proper proportion of potassium to calcium will, over some range of absolute concentration, permit the heart to continue its normal beating. The mammalian analogy is imperfect, since both calcium and potassium in excess lead to arrest in diastole. However, an increase in automaticity occurs during one phase of the action of calcium on the mammalian heart (4), appearing at concentrations of calcium comparable to those noted in table 1. On the other hand the potassium alone has no effects other than depression of cardiac

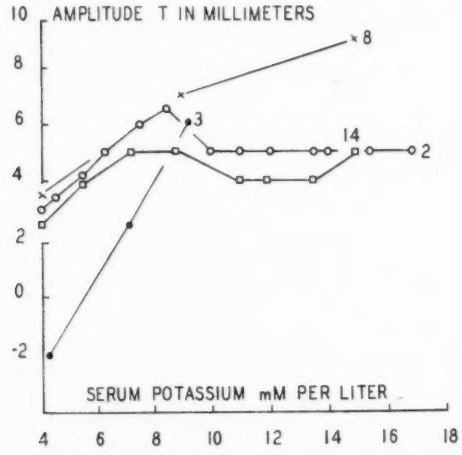


Fig. 3. As figure 2, but showing relationship between amplitude of T wave and serum potassium during injection of calcium and potassium mixture. To reduce the size of the graph, the potassium values in experiment 8 are reduced one-half.

activity (10). When administered together it seems reasonable to suppose that the stimulant effects of the calcium may partially oppose the depressant effects of the potassium, and permit the hearts of these animals to continue contraction at concentrations of potassium which otherwise would cause arrest.

Nicholson and Soffer (5) have suggested that the disappearance of the P waves in the course of potassium injection is due to auricular fibrillation. In order to test this hypothesis, two cats with exposed hearts were followed through the entire course of potassium intoxication. The auricles ceased beating completely some time before ventricular arrest, and electrocardiographs taken at this time showed absence of P waves. The general course of events preceding the disappearance of P waves is consistent with auricu-

lar asystole rather than fibrillation. There is a gradual diminution in height and widening of the complex similar to that occurring in the ventricle, with varying degrees of delay in A-V conduction, while there is no indication of fragmentation of the P wave, flutter, or coarse fibrillation.

Effects of potassium on skeletal muscle. The persistence of vigorous muscle twitches while the muscles are perfused with blood containing potassium in concentration sufficient to produce cardiac arrest indicates clearly the highly specialized character of the toxic effects of potassium on the heart. Arrest of the heart is apparently not due to any profound damage which potassium in fatal concentration exerts on cell metabolism or activity generally, but to a very specific effect on cardiac automaticity and conductivity. The observations of Tennant and Liebow may be cited in this connection (7). They found that the fluid in which isolated embryonic cardiac tissue was cultivated might contain more than 50 mEq. of potassium per liter without producing death or even interference with growth, but that at concentrations higher than about 17 mEq. per liter cardiac automaticity did not appear.

From these experiments it is impossible to determine whether excess of the potassium depresses the contractility of the individual muscle fibers or whether it acts by preventing proper spread of the impulse throughout the cardiac muscle mass. The wide complexes just prior to death suggest that defective intraventricular conduction is important, although toxic effects on muscle may be present as well.

Throughout this discussion the toxic effects have been compared with the concentration of potassium in the serum. Such a treatment is justified since these are the actual experimental variables between which a correlation has been established. However, any understanding of the underlying physiology of the process must include a consideration of changes in tissue potassium as well. This is especially true since it has been shown (9, 11) that intravenously injected potassium distributes itself freely throughout the main muscle mass of the body. It must therefore be assumed that potassium enters the skeletal muscles in the course of these experiments. It is, however, uncertain whether it enters cardiac muscle with equal facility. Evidently the presence of a considerable increase of the potassium in serum and so in the skeletal muscle cells themselves can be tolerated without deleteriously affecting the activity of the skeletal muscles. Whether similar penetration into the heart takes place, and if it does so why it should be so destructive in its effect, remains for future experiments to decide. An excellent example of dissociation between the potassium content of cells and serum concentration of potassium occurs under some circumstances in familial periodic paralysis (6). In this disease paralysis of the muscles is usually associated with a drop of serum potassium; if now potassium is given by mouth it may pass into the muscles and the paralysis be relieved while the concentration of potassium in the serum remains very

low. Another example is adrenal insufficiency, in which the muscle potassium is known to be high (2). Animals in acute adrenal insufficiency are extremely sensitive to the administration of potassium salts. It is hardly allowable to compare directly the toxic effects in normal animals with those in adrenalectomized animals, if only because the potassium content of their tissues and presumably their mode of dealing with potassium in the muscles is definitely abnormal. Nicholson and Soffer (5) found higher concentrations of potassium in the serum of living dogs with acute adrenal insufficiency than are compatible with life in normal dogs. The difference may well be associated with the different state of the tissue potassium.

SUMMARY

1. Potassium chloride in isotonic solution and mixtures of potassium and calcium chlorides in isotonic solution were injected intravenously into dogs, at a slow uniform rate until death occurred. Electrocardiograms were taken at intervals, and initial and final blood samples obtained for potassium and calcium analysis.

2. In nine experiments in which potassium chloride alone was injected death occurred at concentrations of potassium in the serum ranging from 12 to 20 mEq. per liter. The average concentration was about 15 mEq. per liter.

3. In 4 of 9 experiments in which calcium chloride was injected simultaneously with potassium chloride the concentration of potassium in the serum at death greatly exceeded this range. In the remaining five the concentration fell in the upper range of values associated with death from potassium alone.

4. Muscle twitches during the course of the injection of potassium chloride continued vigorously even at concentrations of potassium in the serum sufficient to cause cardiac arrest.

5. The toxic effects of potassium on the heart are due to some highly specific effect on that organ, not shared by skeletal muscles generally.

REFERENCES

(1) Cushny, A. R. A textbook of pharmacology and therapeutics. 10th ed., revised by C. W. Edmunds and J. A. Gunn, x, 786 pp., Philadelphia, 1934.
(2) Darrow, D. C. and H. E. Harrison. J. Clin. Investigation **17:** 77, 1938.
(3) Hald, P. M. J. Biol. Chem. **103:** 471, 1933.
(4) Hoff, H. E., P. K. Smith and A. W. Winkler. This Journal **125:** 162, 1939.
(5) Nicholson, W. M. and L. J. Soffer. Johns Hopkins Hosp. Bull. **56:** 236, 1935.
(6) Pudenz, R. H., J. F. McIntosh and D. McEachern. J. A. M. A. **111:** 2253, 1938.
(7) Tennant, R. and A. A. Liebow. Personal communication.
(8) Thomson, W. A. R. Lancet 808, 1939.
(9) Wilde, W. S. J. Biol. Chem. **128:** 309, 1939.
(10) Winkler, A. W., H. E. Hoff and P. K. Smith. This Journal **124:** 478, 1938.
(11) Winkler, A. W. and P. K. Smith. J. Biol. Chem. **124:** 589, 1938.

THE RANGE AND VARIABILITY OF THE BLOOD FLOW IN THE HUMAN FINGERS AND THE VASOMOTOR REGULATION OF BODY TEMPERATURE

A. C. BURTON

From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania

Received for publication May 20, 1939

The physical regulation of body temperature depends mainly upon the physiological control of the peripheral circulation, when the environmental temperature is below that where sweating plays a major rôle. By adjustment of the peripheral blood flow the effective thermal conductivity of the tissues may be altered over a five or six-fold range of values, as shown by Burton and Bazett (1936) in water baths and by Winslow, Herrington and Gagge (1937) in atmospheric environments.

The regulation of the average flow to the requirements of heat elimination is, of course, brought about by the nervous control of the tone of the peripheral blood vessels. This control is mediated chiefly by the sympathetic system, though a measure of "metabolic" regulation of flow may exist in a denervated limb (Freeman, 1935). An inhibitory action mediated by fibres from the dorsal roots has been demonstrated in the frog by Bozler (1936) but it is not yet clear how much this must be considered in the mammal.

A series of researches in this laboratory on the activity of the sympathetic system has shown that under the influence of afferent stimuli it is in a state of continual fluctuation of tone. The activity of the sympathetic centres may be of a rhythmic and intermittent character which bears no obvious relation to any corresponding intermittence of afferent influences (Bronk, 1936). The activity of the cardiac sympathetic nerves (Bronk et al., 1936) shows the interaction of several rhythms of various origins. The setting of the average peripheral blood flow to the level required by the demands of temperature regulation must then be made by the modification of an underlying sympathetic tone which itself has a complicated pattern of activity.

Another complicating factor is that the regulation of peripheral flow to serve the function of elimination of body heat must proceed simultaneously with other types of regulation of blood flow. Among these are the supply of oxygen in accordance with the needs of local metabolic activity, and the

counteracting of changes in blood pressure and flow which might otherwise occur in tissues where constancy is of more vital importance.

It is of interest, therefore, to study in detail the range and fluctuation of blood flow in peripheral vessels, in order to obtain a clear picture of the operation of these factors in the control of body temperature. For this study the fingers were chosen for two reasons. They are richly provided with arterial-venous anastomoses (Clark, 1938), the function of which presumably is to effect large changes in the amount and distribution of the blood flow. The fingers also are practically free from muscular tissue. Since Friedlander et al. (1938) find that the physiological changes in blood flow in the muscles may be of opposite sign to those simultaneously occurring in the skin, this is an advantage in simplifying the interpretation of the changes of blood flow of the finger.

Apparatus and method. The method of Hewlett and Van Zwaluwenburg (1909), which has been used by many investigators on the foot, the leg, the hand and the arm (see Freeman, 1935) has been adapted for the measurement of flow in the finger. This adaptation has been briefly reported (Burton, 1938) and a paper has recently appeared by Wilkins, Doupe and Newman (1938) in which a very similar technique was used.

The principle of the method of flow measurement is that, if the outflow of blood from a finger is suddenly arrested by occlusion of the digital veins, without interference with the inflow, the initial rate of increase of the finger volume measures the rate of inflow of blood at the moment of occlusion.

The finger plethysmograph (*A*, fig. 1) is a light metal cylinder covering the last two phalanges of the finger, to which it is sealed by a binding of gauze over zinc oxide paste. It is a great advantage to have a light plethysmograph carried freely by the finger, rather than one rigidly clamped to the bench. With the latter, every movement of the hand results in an artefact on the record, while the former is practically free from this even when no restraint is applied to the hand. Air transmission may be used instead of the customary water system, for temperature changes in a system of so small a volume cannot possibly take place rapidly enough to vitiate the flow determinations which occupy a few seconds only. The recording system consists of a capsule (*C*, fig. 1), about one inch in diameter, covered with a membrane of the thinnest rubber tied on under so slight a tension that a considerable increase in volume may take place with a negligible rise in pressure. A mirror chip (*M*) cemented at the periphery gives optical recording on the bromide paper of a recording camera. Plethysmograph and capsule are connected by pressure tubing. Where long distances of transmission are required, metal or glass tubing is used to avoid distortion of the wave-form of the pulsations. Calibration of volume change is easily made by substitution of a 1 cc. syringe for

the plethysmograph. Figure 2 *A* is the record of a sudden driving in of the plunger of the syringe and shows that the system is rapid in response and gives no sign of overshoot due to inertia.

Occlusion of the venous outflow is made by a small cuff (*D*, fig. 1) placed round the remaining phalanx of the finger. This is made of "drainage tubing" backed by adhesive tape. A bypass tape (*E*, fig. 1) is conveniently included in the system so that the volume may be returned to the baseline value after any considerable change, such as produced when the finger is being inserted in the plethysmograph or immediately before a flow determination.

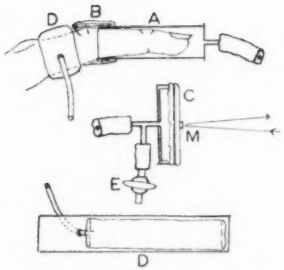


Fig. 1

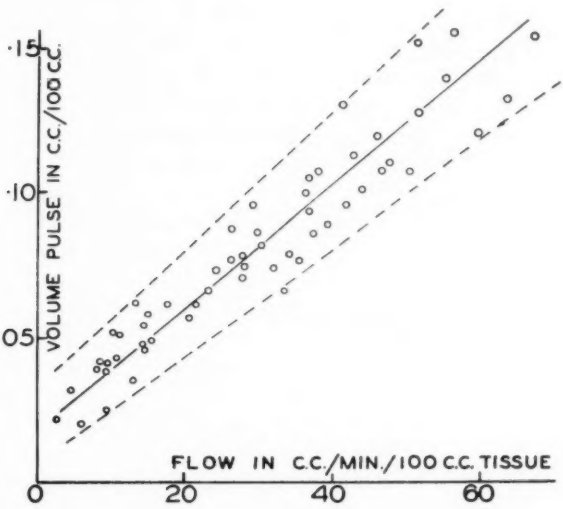


Fig. 6

Fig. 1. Apparatus for measurement of blood flow in the finger
Fig. 6. Relation of size of pulsation in a finger to blood flow

Figure 2 shows typical records of flow determinations made at three different levels of blood flow. No signal is necessary to mark the point at which the occlusion became effective, as inspection of the shape of successive pulsations shows this point more accurately than any signalling device that could be devised. When the flow is in the low range (*D*, fig. 2) the diastolic volume rises linearly for many beats and there is no difficulty in estimating the initial slope. With higher flows (*C*, fig. 2) the increase of volume soon becomes less rapid and after ten or twelve beats the pressure in the finger vessels has risen sufficiently to make the outflow past the inflated cuff once more equal to the inflow, so that no further volume in-

crease occurs. With still higher flows (B, fig. 2) more care must be exercised in drawing the initial tangent to the curve of diastolic volumes. For the highest flow a special construction must be used to find the virtual diastolic volume at the moment of occlusion (fig. 3). The point A is obviously that at which occlusion became effective. The sudden rise AB is a "cuff artefact" which is often present due to the pushing of tissue into the plethysmograph by the inflation of the cuff. The height AC indicates

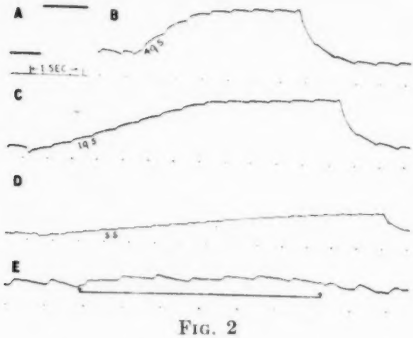


Fig. 2

Fig. 3

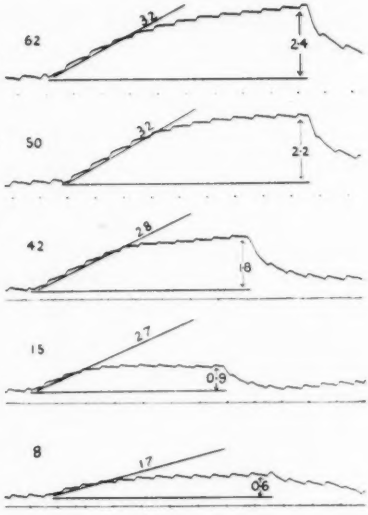


Fig. 4

Fig. 2. A, rapidity of response of the recording system. B, C and D, flow determinations in the ranges of high medium and low flow respectively. E, case of very high flow where the determination is not possible. Time in sec.

Fig. 3. Geometrical construction used when the flow is very great.

Fig. 4. Effect of different occlusion pressures. Numbers on the figures indicate, from left to right, the occlusion pressure in millimeters of mercury, the flow in cc./min./100 cc. indicated by the initial slope, and the total increase in finger volume in cc./100 cc. Time in sec.

the level of the volume above the diastolic volume at the moment of occlusion. A vertical is dropped from B to D, so that BD is equal to AC. D is the starting point of the curve to be drawn through successive diastolic volumes. The estimate of the initial slope in such cases is admittedly subject to error, but repeated constructions on the same record gave correspondence of the estimated flows within 10 per cent even in such an extreme case as that of figure 3. In certain cases, notably in the sclerodermic subject and in the normal at the upper limit of possible flow, the method is

not applicable. Here the distensibility of the vessels has become very slight, and the vessels are full to their limit, so that upon occlusion the total increase of volume is very small (fig. 2, *E*). All that is recorded here is a notable decrease in the size of the pulsation. In such cases the size of the pulsation before the occlusion is the indication that the flow is very great.

Figure 4 shows that over a wide range the occlusion pressure used in the cuff has little effect on the initial slope of the curves, i.e., on the measured value of the flow. Cuff pressures below 15 mm. Hg are likely to give estimates of flow that are erroneously low, due both to difficulty in drawing the initial tangent and to incomplete occlusion of the veins. Higher pressures than 55 mm. Hg again tend to give values that are too low, here because of interference with the inflow of blood. Between these limits repeated determinations give values which scatter considerably, due to physiological variation, but which show no systematic relation to the occlusion pressure. The total volume increase of the finger before constancy is reached, however, increases with the pressure, since more blood has to flow into the vessels before the pressure has risen sufficiently at the cuff for outflow to equal inflow.

No evidence could be found that repeated venous occlusion and release at intervals of 10 seconds or less gave any systematic change in the blood flow of the finger, though with the hand or the arm where longer times of occlusion are necessary this may be a source of error.

Results. *Range of the blood flow.* In some 500 determinations of the flow in the fingers of seven normal subjects a range of from 1 cc./min./100 cc. of tissue up to a maximum of about 90 cc./min./100 cc. tissue was encountered. These experiments included the administration of constrictor and dilator drugs and the use of extremes of temperature, both local and general. Even in cases of Reynaud's disease with maximum constriction of the blood vessels, flows less than 0.5 cc./min./100 cc. tissue were not found. Average values over a five minute period lying anywhere between 15 and 40 may be taken as normal for the subject who is comfortable as regards the temperature of the surroundings. Dilatation produced locally by immersion in water at 44°C. or reflexly by heating for 30 minutes with hot pads on the legs gave a maximum flow usually between 80 and 90 cc./min./100 cc. In certain cases, however, even this enormous flow of blood may be exceeded. In a subject living for several days in a hot and humid room (dry bulb temperature 32.4°C., wet bulb, 26.3°C.) the maximum flow was found to increase during five days of acclimatization from 96 to 122 cc./min./100 cc. In the following period in a cold room (dry bulb temperature 21.1°C., wet bulb, 16.0°C.) the minimum flow measured decreased in three days from 16 to 1.8 cc./min./100.[1] The limits of

[1] Details of these results will be given in a paper with H. C. Bazett and J. C. Scott, in collaboration with whom the experiments on acclimatization were made.

the range of flow in the fingers therefore are variable according to the degree of acclimatization to the environment, and summer and winter values would be expected to differ.

Determinations on different fingers of the same subject, and on the fingers of different subjects where the volume of the finger differed greatly gave similar values for the range of blood flow when expressed per 100 cc. of tissue.

Fluctuation of the blood flow. Successive determinations of flow made only a few seconds apart seldom give values which approach equality (fig. 5 A). They may indeed differ by as much as 50 per cent of their average value (fig. 5 B). Accompanying such changes in the indicated flow there is a corresponding change in the size of the volume pulsation, and also in the finger volume (the "baseline" of the pulsations). There is

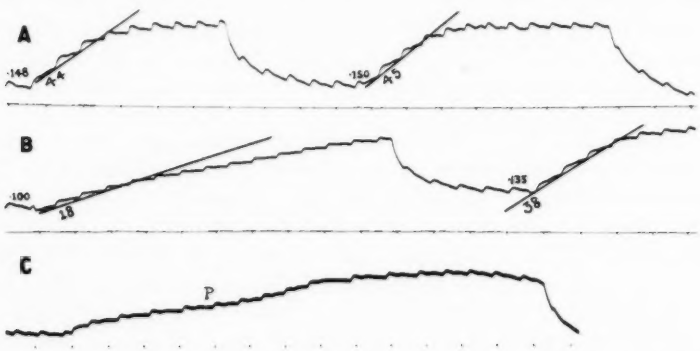


Fig. 5. Size of pulsation and flow. In *A* both are constant, in *B* both are rapidly increasing. *C* shows a case where the flow is changing during the determination itself. Time in sec.

a very close correlation between the computed values of the flow and the size of the volume pulsation at the moment when the determination was made. Figure 6 shows the extent of this correlation for measurements on a single subject. Here the correlation coefficient is 0.88 ± 0.015. In view of this it does not seem possible that the apparent fluctuations of flow are the result of large errors of measurement. They must indicate, rather, a very great physiological variability in the blood flow of the finger.

The flow may change so rapidly that in a determination the curve of rise of volume during venous occlusion has an unusual shape as in figure 5 C, which shows a record made on a subject having a very marked cardiac arrhythmia in the respiratory cycle. Figure 7 shows the result of a series of flow determinations every 10 seconds on the same subject, lying quietly and comfortably. Three series of determinations are plotted in

which the average flow was in the low, medium and high ranges. It will be noted that there are "waves" of change, the amplitude of which is greatest in the middle range of flow. In the low range, i.e., in constriction, the flow remains relatively constant. In dilatation the tendency is to remain high with occasional brief decreases in flow. The figure suggests that the fluctuation is not entirely random in its nature, but may be a complex result of several rhythmic components. Flow determinations cannot well be made at intervals more frequent than every six seconds, and are inadequate to determine whether this is so. For the detailed analysis of the fluctuations another method, however, is available, for making use of the close correlation of figure 6, the size of successive pulsations may be used as an index of the fluctuations of vasomotor activity.

Analysis of the fluctuations. Figures 8 A and 8 B show that the changes in finger volume and in size of pulsation respectively are indeed of a rhyth-

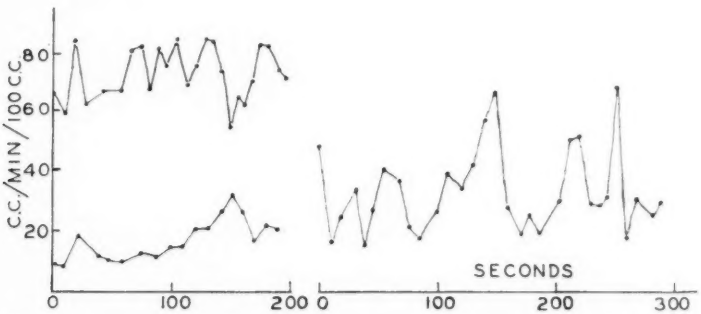


Fig. 7. Fluctuations in flow in the finger at three levels of the average flow

mic character. Figure 8 C is the result of detailed measurement and plotting of the size of pulsation in records such as figure 8 B. The complexity of the changes is due to the presence of at least two types of rhythmic fluctuation. The first of these is the well known fluctuation of flow in the respiratory cycle, studied by Bolton, Carmichael and Sturup (1936) and by Shulman, Mulinos and Lieb (1938). Simultaneous records of the respiration proved the respiratory origin of the waves of small amplitude and period seen at *R* in figure 8 C. In most subjects the amplitude is such that a variation of some 20 per cent in flow is indicated in each respiratory cycle. In a subject, otherwise normal but having a very marked cardiac arrhythmia of respiratory nature, whose cardiac rate swung between 45 and 85 per minute in each respiratory cycle, the fluctuation in flow amounted to 60 per cent. Even in the normal, the inflation of the lungs by a deep breath causes a momentary almost complete cessation of flow in the fingers.

The second component consists of much slower waves of large amplitude, marked *X* in figure 8 C. Their period varies from as little as 15 seconds to as much as 120 seconds, the longer periods being associated with a high average flow. Characteristically in this case the shape of the fluctuation (fig. 8) indicates a rapid constriction followed by a slower recovery of dilatation. Since there is such a marked vasoconstriction from a deep breath, a simultaneous respiratory record was examined in one case to

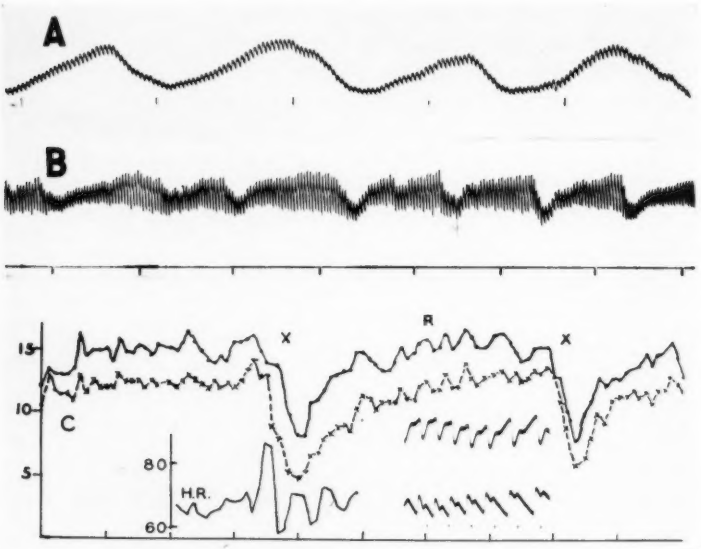


Fig. 8. Rhythmic fluctuations in the finger vessels. *A* shows the changes in finger volume; *B*, taken with a leak in the recording system, shows changes in size of pulsation. *C* is a plot of measurements of the size of pulsation in a finger of the right hand (solid line) and in the left big toe (broken line). Time units, 30 secs. in A and B; 20 secs. in C.

make sure that the large rhythmic constrictions were not related to periodic "sighing" of the subject. No such relation was seen.

Though these slow waves were found in all records examined, their amplitude and frequency varied greatly in different individuals under the same conditions of temperature. Figure 9 shows records taken with a slower camera speed on four different normal subjects. Repeated measurements on different days indicated the same differences in relative amplitude of the waves, which must, therefore, reflect a difference in the stability of the sympathetic system in these individuals.

A third component of the fluctuation that may be present is the con-

striction in response to the stimulation of pain, startle or emotion. For instance, at the points marked by arrows in figure 9, an unexpected noise was made behind the subject. The resulting constriction is plainly seen. Even the mere asking of a question of the subject sometimes resulted in such a response. This type of response was strikingly seen also in a series of experiments with Dr. Hugh Montgomery on the effect of drugs upon blood flow in the fingers in which a subcutaneous injection of saline or of distilled water was routinely used as a control during the course of the experiment. This invariably produced a constriction (fig. 10). It will be noted that constriction preceded by some seconds the actual insertion

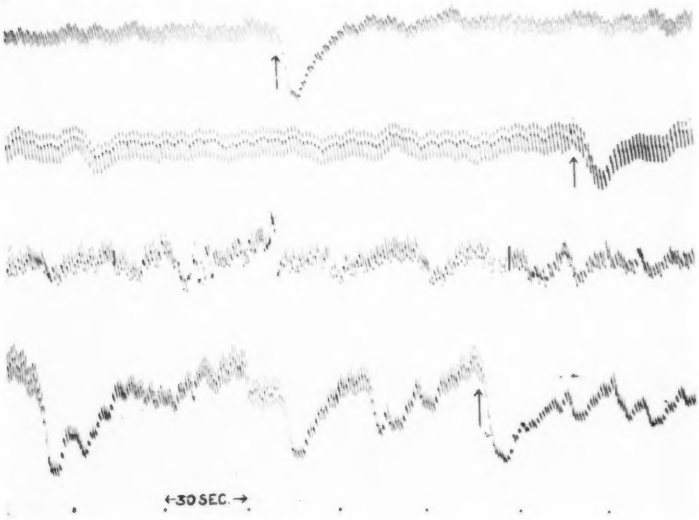


Fig. 9. Extent of fluctuations in different subjects at the same temperature. The arrows mark the times of an unexpected noise.

of the needle of the syringe, which produced a slight further effect. The mere anticipation of an injection was sufficient to produce a constriction. The peripheral blood flow is a much more sensitive index of "psychic" constrictions than the blood pressure or cardiac rate, which may simultaneously show little significant change.

Fluctuation in different parts of the body. Simultaneous records have been made of the volume pulsation in two fingers of the same hand, in a finger and in one of the opposite hand, and of a finger and a toe volume pulse. The fluctuations are closely correlated in parts of the body as widely separated as a finger of the right hand and the left big toe. The extent of the correlation is shown by figure 11. The demonstration of a

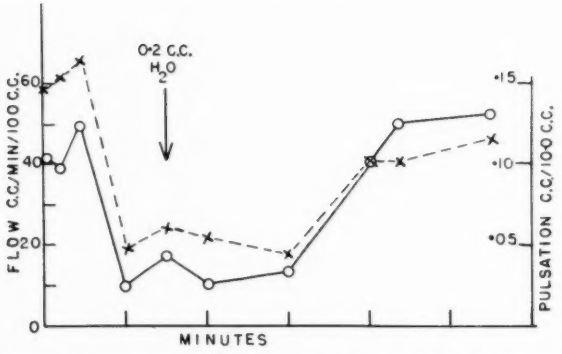


Fig. 10. Changes in pulsation and in flow in a finger induced by the anticipation and the administration of a subcutaneous injection.

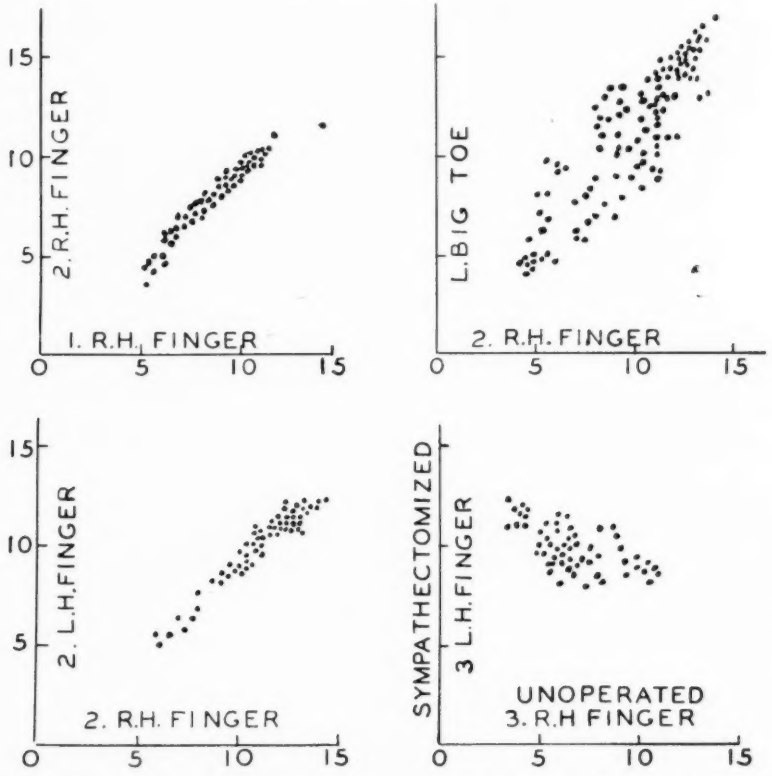


Fig. 11. Correlations between simultaneous values of the pulsation in different digits.

bilateral symmetry of the changes is practically as perfect as the errors of measurement would allow. Between the changes in finger and toe there is more scatter in the correlation diagram, but this occurs mostly in the middle range, and is due to the fact, as inspection of figure 8 shows, that when the flow is changing rapidly a slight time difference in the changes will have a major effect. There is no doubt that the rhythmic changes in blood flow are simultaneous over a large part of the peripheral vascular bed.

Fluctuations of cardiac rate and blood pressure. With each of the large periodic constrictions of figure 8 there is a simultaneous increase of cardiac rate (which could be measured for each beat from the records). An example is shown on figure 8 C (*H.R.*) and one in figure 12. The cardiac change is complicated, however, by a subsequent slowing due no doubt to

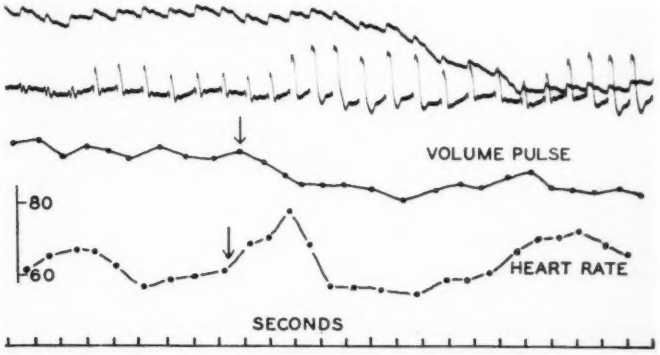


Fig. 12. Changes in blood pressure and in heart rate during a periodic constriction

the operation of the carotid sinus and aortic arch reflexes, so that the records of heart rate show diphasic changes where the volume pulse records are monophasic. There is then a discharge of impulses in the cardiac sympathetic nerves, or an inhibition of vagal impulses, simultaneous with the peripheral constrictions. It is interesting to note that though the heart is markedly slowed the progress of the constriction is not halted.

It is obvious that widespread simultaneous changes of the peripheral vessels over the body must produce changes in blood pressure. The latter is not readily measured with sufficient rapidity in the human for the purpose of finding the amount of these. It may be shown, however, that the changes in pressure occur, and are of the opposite sign to the blood flow changes (fig. 12). If the pulsation of the brachial artery is recorded from a small bag under a Riva Rocci cuff held constant at a pressure just below the systolic pressure, "spikes" appear whenever the blood

pressure rises above that of the cuff. It is obvious from figure 12 that there is a rise of systolic pressure simultaneous with a decrease in the pulse volume recorded from a finger on the opposite side of the body. (The time lag in the volume change over that of pulse-volume may be noted.) By holding the cuff at about the diastolic pressure, changes in the latter may be followed by the appearance and disappearance of the flattening of the waves at the end of diastole or by other diastolic criteria. In the large waves of constriction, diastolic pressure as well as systolic rises, but in the fluctuations of smaller amplitude, such as the respiratory waves, the relation is very complicated. Diastolic pressure may here be rising as systolic falls, probably because of the complicating factor of change of cardiac rate.

Fluctuations in a sympathectomised limb. Simultaneous records were made of the volume pulsations in a finger on the unoperated and on the operated side of a patient in whom a unilateral cervical ganglionectomy had been done some months before the experiment. The resulting correlation diagram is shown in figure 11. While the size of pulsation on the unoperated side varied between the usual limits, the pulsation of the sympathectomised side had a much smaller range of variation and remained close to the value found in a normal dilated limb. Correlation between the pulsation on the two sides is small, but it is clear that any that exists is of the opposite sign to that found between normal digits. The pulsation of the sympathectomised digit tends to increase when that of the normal digit is small. Thus its fluctuations are in phase with those of the blood pressure changes which accompany the widespread vasoconstrictions of the normal vessels and may be attributed to a passive dependence upon the mean blood pressure. The experiments with the sympathectomised subject show that the occurrence of normal rhythmic changes of flow in a limb is dependent upon an intact peripheral sympathetic system.

Modification of the rhythms by temperature regulation. The modification of the amplitude of the fluctuations by the temperature conditions has already been mentioned. When the subject is cold and the average blood flow is low the fluctuations are small in amplitude. In conditions of full dilatation when the subject is uncomfortably warm and sweating, it is again less (fig. 7). Here the constrictions, which still occur at intervals, are often slight so that the size of pulsation does not fall far below the maximum dilatation value.

Temperature regulation also modifies the frequency of the periodic constrictions. Statistical studies of the intervals between successive constrictions that occur in the course of a long experimental period (80 min.) have been made, in collaboration with Dr. R. M. Taylor, at different environmental temperatures. In the same subject the average period

increased from 50 seconds at 24°C. to 80 seconds at 30°C., while the period of commonest occurrence increased from 30 seconds to 50 seconds. Within an experimental period of constant temperature the rhythm is often seen to pass from a slow to a faster tempo for a short period. In such periods of faster rhythm the pulsation never reaches the full dilatation value between constrictions, so that the average pulsation, and therefore the average flow, decreases.

Both the frequency of the waves of constriction and the magnitude of the contractions of the blood vessels are therefore modified by the integrated sum of afferent stimuli from temperature receptors.

Discussion. The extraordinary range of blood flow in the fingers, permitting a hundred-fold change between minimum and maximum values, must be related to their functions as radiators of body heat (Grant and Pearson, 1938) since such a range cannot represent a provision for metabolic needs. The range found in the hand is less (Freeman, 1935) from a slightly higher minimum (1 cc./min./100 cc.) to about 20 cc./min./100 cc. The similarity of the minimum values suggests that these represent the basic requirement of blood flow for the maintenance of the metabolism of the skin. Field, Belding and Martin (1938) give values for the oxygen consumption of the various tissues of the rat (0.3 cc./hr./gm. wet weight for skin). If it may be assumed that the relative metabolic activity of different tissues is the same in man as in the rat, a value for human skin may be deduced by multiplying the value for the skin of the rat by the ratio of the total metabolism per gram in the two cases (0.2 for man, 1.1 for the rat). Taking the values for available oxygen in the blood in severe ischemia given by Freeman, Shaw and Snyder (1936), a simple calculation yields as the minimum requirement of blood flow the value 0.8 cc./min./100 cc. tissue, agreeing closely with that found in full constriction. For the hand, because of the presence of muscle, the minimum requirement would be greater.

Analysis of the fluctuations of flow reveals that the heat regulation is not achieved by the setting of the peripheral flow to an appropriate steady level except at the ends of the range of adjustment. In the middle range of flow it is achieved rather by the modification of an underlying intermittence of flow between high and low values; an intermittence which reflects the rhythmically fluctuating character of the tone of the sympathetic system. A crude analogy might be drawn with the operation of a temperature regulated water-bath, where the constancy of the average temperature is accomplished by the adjustment of the relative durations of the "on" and "off" periods of the heater.

The increase in the frequency of the discharge of sympathetic bursts as the temperature of the environment is lowered should probably be regarded as the primary mechanism of control of the average blood flow,

though modification of the number of impulses in each burst undoubtedly also plays a part in the grading of the contractions of the smooth muscle. Bozler (1936) shows that following a brief stimulus of the sympathetic nerves the relaxation of the blood vessels of the frog's limb may not be complete for two minutes. The sluggishness of the response of the effectors supplied by the sympathetic system in the mammal has been discussed by Adrian, Bronk and Phillips (1932). It is to be expected then that with rapidly occurring bursts, as in cold conditions, the result would be a maintained state of contraction of the vessels, with only partial relaxation between the bursts. The diminished amplitude of the fluctuation of flow in the cold conditions may thus be a consequence of the slowness of response of the effectors.

The existence of rhythmic fluctuations is of great interest on its own account. The simultaneous widespread nature of the constrictions and the accompanying cardiac acceleration shows that the sympathetic system is discharging as a whole, driven by a physiological centre, a conclusion reached also by the study of the electrical activity of the sympathetic nerves (Bronk, 1934). The faster rhythms have previously been known and studied. Bronk et al. (1936) studied both the cardiac and respiratory rhythms as well as others of different frequency. The repiratory constriction has been studied recently by Bolton et al. (1936) and by Shulman et al. (1938). The much slower rhythms here discussed are linked by the existence of accompanying blood pressure changes to the Traube-Hering or Mayer waves (Traube, 1865; Hering, 1869; Mayer, 1876; Irving, 1938). Some have regarded these as representing the activity of a failing vasomotor centre, as gasping respiration does that of a failing respiratory centre, for in anesthetised animals they are not constant in occurrence (Alkjaer, 1934; Speigel and Taga, 1930). Alkjaer (1937), however, finds slow waves always present in the blood pressure of unanesthetised humans, though suppressed in psychic constriction. Wilson (1936a) studying the visible changes in blood vessels of the ear of rabbits and dogs showed that they were synchronous with blood pressure changes, and, moreover, that the waves were suppressed by the common anesthetics (Wilson, 1936b). In the unanesthetised rabbit they were always present as long as the animal was awake. In humans, unless in a state of constriction, the waves have invariably been seen in the experiments. Rhythmic fluctuations in vasomotor tone are then rather to be regarded as normal in the unanesthetised animal. The phenomenon is then not of casual interest but of fundamental physiological importance.

It is as yet uncertain whether it is wholly justifiable to speak of the rhythmic vasomotor changes as truly "spontaneous." Doupe et al. (1937) found that "spontaneous" changes of toe volume did not occur in patients with lesions of the cauda equina, and concluded that the vaso-

constrictions normally seen resulted from afferent impulses from the legs, possibly consequent to periodic venous engorgement. It has been noted in these experiments that the volume changes of the digits are to some extent independent of the flow changes, as though the venous system, which greatly affects the volume in distinction to the volume pulsation, acted independently of the arteriol-artery system controlled by the sympathetic (Turner et al., 1937). Possibly the periodic constrictions of the venous system are due to afferent stimulation and reflex action while the arteriolar constrictions are truly spontaneous. It may be noted also that in the experiments of Doupe and his co-workers the subjects were held as far as possible in a state of maximum dilatation in which spontaneous constrictions would be expected to be absent or very infrequent. The widespread distribution of simultaneous waves of constriction certainly are difficult to explain on the grounds of afferent impulses from local venous engorgement. Also if this were the explanation, a greater frequency of the constrictions would be expected with a greater average blood flow, which would more rapidly fill the veins. The relation between frequency of constrictions and the average blood flow, however, is the reverse, the rhythmic constrictions being less frequent in dilatation than with lower flow.

A second possibility must be examined. Temperature regulation might not merely modify the rhythm but be the cause of the rhythmicity. After a period of high blood flow, the skin temperature might fall due to increased loss of heat until afferent impulses from cold receptors reached a threshold, below that of sensation, yet sufficient to stimulate the centre to initiate a constriction. This possibility cannot be dismissed until a careful study has been made of the relation between the rhythmic fluctuation of flow and skin temperatures.

The accurate adjustment of the average value of so fluctuating a flow in the peripheral vessels, to a level which ensures the measure of constancy of body temperature achieved by the homeotherm, is one of the most highly developed integrative mechanisms of the nervous system.

SUMMARY

A simple plethysmographic method of recording the volume pulsations of the finger has been adapted to the measurement of instantaneous values of the blood flow of the finger in cc./min./100 cc. of tissue. The measurement may be repeated at intervals as short as six seconds.

The range of blood flow in the fingers is from 0.5 to 1.0 cc. as a minimum up to 80 to 90 cc./min./100 cc. tissue for full dilatation. The maximum and minimum values are subject to change upon slow adaptation to high or low temperatures. The minimum value found is shown to correspond with that calculated from the basal oxygen consumption of skin. The

tremendous range of flow, made possible by the arterial-venous anastomoses, represents not a metabolic need of the tissues but the mechanism for temperature regulation by elimination of body heat.

A close correlation is found between the size of the finger volume pulse in cc./100 cc. and the blood flow in cc./min./100 cc. of tissue. This has been used in a study of the nature of the marked fluctuations in the flow which occur from moment to moment.

These are rhythmic in character, the rhythm having two main components, a respiratory fluctuation of small amplitude and a slower rhythm of periodic constrictions of large amplitude. These are simultaneous in the digits of all the extremities of the body and are accompanied by cardiac acceleration and rise of blood pressure. A rhythmic fluctuation of the activity of the entire peripheral sympathetic system acting as a whole is therefore indicated. The connection with Traube-Hering or Mayer waves of blood pressure is pointed out. A third component of constrictions due to pain, startle or emotional stimuli may also be present.

The rhythm is markedly modified by the conditions of temperature regulation. The amplitude of the waves is greatest in the middle range of blood flow and of temperature, is less in dilatation, and least in constriction. The frequency of the rhythm is also modified, the faster rhythms being associated with cooler conditions and with lower average values of the flow.

Physical regulation of body temperature is accomplished, not by the setting of the peripheral blood flow to an appropriate steady level, but by the adjustment of the average of a flow which is fluctuating rhythmically between high and low values.

I am greatly indebted to Dr. D. W. Bronk and Dr. H. K. Hartline for criticism and suggestions in the preparation of this paper.

REFERENCES

Adrian, E. D., D. W. Bronk and G. Phillips. J. Physiol. **74:** 115, 1932.

Alkjaer, V. Skand Arch. Physiol. **69:** 167, 1934; Skand. Arch. Physiol. **76:** 162, 1937.

Bolton, B., E. A. Carmichael and G. Sturup. J. Physiol. **86:** 83, 1936.

Bozler, E. This Journal **117:** 457, 1936.

Bronk, D. W. Harvey lectures, 1934. Cold Spring Harbor Monogr. **4:** 170, 1936.

Bronk, D. W., L. K. Ferguson, R. Margaria and D. Y. Solandt. This Journal **117:** 287, 1936.

Burton, A. C. This Journal **123:** 29, 1938.

Burton, A. C. and H. C. Bazett. This Journal **117:** 36, 1936.

Clark, E. R. Physiol. Rev. **18:** 229, 1938.

Doupe, J., J. S. M. Robertson and E. A. Carmichael. Brain **60:** 281, 1937.

Field, J., H. S. Belding and A. W. Martin. This Journal **123:** 64, 1938.

Freeman, N. E. This Journal **113:** 384, 1935.

FREEMAN, N. E., J. L. SHAW AND C. SNYDER. J. Clin. Investigation **15:** 651, 1936.
FRIEDLANDER, M., S. SILBERT, W. BIERMAN AND N. LASKEY. Proc. Soc. Exper. Biol. and Med. **38:** 150, 1938.
GRANT, R. T. AND R. S. BRUCE PEARSON. Clin. Science **3:** 119, 1938.
HERING, E. Wien. Sitzungsb. **60:** 829, 1869.
HEWLETT, A. W., J. G. VAN ZWALUWENBURG AND M. MARSHALL. Heart **1:** 87, 1909.
IRVING, L. Am. J. Med. Sci. **196:** 888, 1938.
MAYER, S. Wien. Sitzb. **74:** 281, 1876.
SHULMAN, I., M. G. MULINOS AND C. C. LIEB. This Journal **123:** 185, 1938.
SPIEGEL, E. A, AND KEN TAGA. Ztschr. ges. Exper. Med. **70:** 494, 1930.
TRAUBE, L. Centralb. Med. Wissensch. **3:** 881, 1865.
TURNER, R. H., G. E. BURCH AND W. A. SODERMAN. J. Clin. Investigation **16:** 789, 1937.
WILKINS, R. W., J. DOUPE AND H. W. NEWMAN. Clin. Science **3:** 403, 1938.
WILSON, H. C. This Journal **116:** 295, 1936a.
J. Pharmacol. Exper. Therap. **56:** 97, 1936b.
WINSLOW, C.-E. A., L. P. HERRINGTON AND A. P. GAGGE. This Journal **120:** 1, 1937.

THE RADIATING POWER OF HUMAN SKIN IN THE INFRA-RED

JAMES D. HARDY

From the Russell Sage Institute of Pathology in affiliation with the New York Hospital and Department of Medicine, Cornell University Medical College, New York

Received for publication July 3, 1939

During the past three years the question of the emissivity value for human skin has been reopened by Christiansen and Larsen (1) and by Büttner (2). The former authors give values for the emissivity as low as 0.83 and claim that E (emissivity) varies from this value to 1.00 for the "congested skin." Büttner objects to this point of view and estimates experimentally the value, 0.954. In the past it has been taken (3) that E = 98 per cent to 100 per cent, and some measurements (4) made in 1934 lead me to conclude that this assumption is correct. The importance of the knowledge of the precise value of E can be seen from the fact that the difference between 0.954 and 0.98 may cause an error of 0.6°C. in reading skin temperature by the radiation method. The emissivity of the skin is also a factor of importance to air conditioning engineers and students of bio-climatology. It is well known that the emissivity of the skin varies according to the spectral range, but as the skin actually emits only in the infra-red spectral region, from 5μ to about 20μ, it is the emissivity value in this range which is of interest.

There are three possible ways to measure the emissivity of the skin. Either the reflecting power or the absorbing power of the skin in the wave length range from 5μ to 20μ will determine the emissivity, or the emitting power may be measured directly, providing the skin temperature be known. From the well known relationship of Kirchhoff the emitting power of the skin for a given temperature and wave length range may be compared to that of a black body. The relationship is

$$S = \frac{E}{A} = 1 = \frac{E}{1 - R} \tag{1}$$

where S = radiating power of a perfect black body, E = emissivity, A = absorbing power, and R = reflecting power.

Thus E is determined if either A or R be known. So far as I am aware no measurements of A have been made in the spectral range from 5μ to 20μ, and it would be difficult technically to attain a calorimetric precision high enough to make a precise determination of A. The more common

procedure is to make measurements of the reflecting power. The reflection of the skin has been extensively studied in the visible and ultra-violet spectral regions although little attention has been paid to the infra-red regions beyond 3μ. Hardy and Muschenheim (5) compared the reflection of the skin with that of $MgCO_3$ from 1μ to 10μ. No other measurements of the reflecting power of the skin in the infra-red have been made although Büttner gives a composite reflection curve of skin, representing the work of many authors, from 0.3μ to 50μ. This curve is in agreement with Hardy and Muschenheim as far as 6μ.

The other method for measuring E is to compare the emitting power of the skin with that of an experimental "perfect black body" on the basis of of the Stefan-Boltzmann law. This well known relationship is

$$A = E\,E'\,k\,(T_e^4 - T_s^4)$$

where A is the radiation exchange, E' = emissivity of the emitter, E = emissivity of the skin, T_s = skin temperature, and T_e = temperature of the emitter.

This equation has been applied by Aldrich (6), Cobet and Bramigk (7), Hardy, Christiansen and Larsen, and Büttner. Values from 0.83 to greater than unity have been obtained by these authors. Usually the value of E has been taken as so nearly unity as to require no correction to the Stefan-Boltzmann formula.

It is the purpose of the paper to present new estimations of E from reflection values and from emissive power determinations.

I. *Reflecting power of skin in the non-penetrating infra-red spectral region.* The experimental arrangement for measuring the total reflection from the skin was an adaptation of the method used by Hardy and Muschenheim to measure the transmission of scattered light through skin, and is shown in figure 1.

The area of the skin tested was the central portion of the forehead. The subject's head, H, was held to the aperture A in such a manner that 40 sq. cm. of skin area were exposed. A was mounted on a vertical axis in the center of a graduated circle. A horizontal bar to which was fastened the radiometer, R, was attached to the circle so that the radiometer could be swung through all angles from 0° to 90°. The radiometer pointed always at the center of A. The source of radiation, E, was placed normal to the aperture at a distance of 60 cm. or more. Between the aperture and the emitter was placed a sliding shutter, S, of asbestos overlaid with polished copper. The skin could be exposed to the radiation from the emitter for any desired time by lowering the shutter.

Two separate sources of radiation were used. An electric stove which could be heated to about 400°C. was used for the shorter infra-red region. The spectral distribution of the radiation from the stove was determined

and is shown in figure 2 (curve *1*). The other source of radiation was in reality a "sink" for radiation and consisted of a block of CO_2 20 cm. x 20 cm. The actual source of radiation in this case was the radiometer itself. The spectral distribution of the radiation of the radiometer to the CO_2 is also shown in figure 2 (curve *3*), together with the emission curve of the skin to an environment at 20°C. as computed from Plank's law (curve *2*).

In order to apply Kirchhoff's law to the reflecting power of the skin and arrive at a determination of E in the spectral range in which the body radiates, it is necessary to test the reflecting power in that range from a

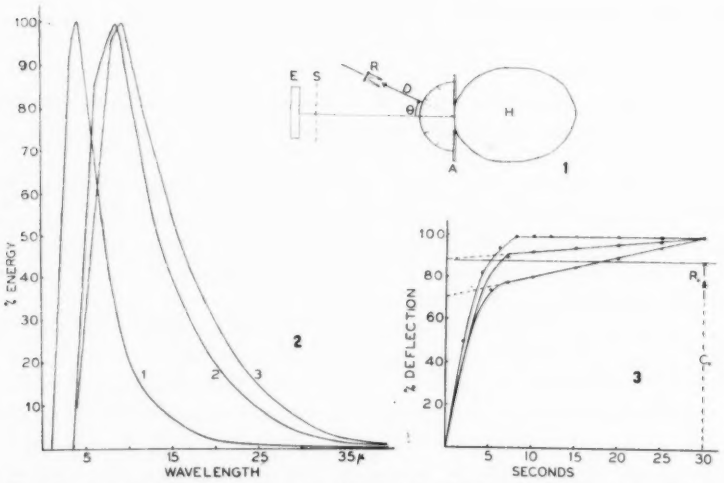


Fig. 1. Arrangement for measuring the total reflection from the human skin.

Fig. 2. Emission curves of 1, hot stove; 2, human skin; and 3, radiometer at 25°C. to CO_2.

Fig. 3. Method of measuring R_θ.

body which has the same spectral distribution of energy as the warm skin. It is not possible to attain this condition exactly but with the CO_2 it is closely approximated as is seen from figure 2. It is evident that the hot stove does not even remotely approximate the necessary situation as it radiates considerably in the region of 1μ where the skin does not radiate. The reflecting power of the skin for the radiation from the stove is included here mainly as a control experiment.

The experimental procedure was as follows: the subject placed his head in position and remained quiet for some minutes. The radiometer was moved to the smallest angle possible from the normal, which in the present case was 30°. The shutter was lowered and the galvanometer

deflection observed for 30 seconds of exposure. After 30 seconds the shutter was raised and the skin temperature observed for 30 seconds longer. The same procedure was followed with a smooth block of $MgCO_3$ (and also a roughened block). The reflection of a piece of silvered ground glass was also measured. Finally the reflection from the sides and surfaces of the aperture were measured as a correcting factor. This procedure was repeated at 40°, 50°, 60°, and 70° and on the other side of the normal. The intensity of the incident beam was then measured with a radiometer.

The amount of energy reflected from the surface under measurement at any angle can then be easily estimated as

$$R_\theta = (I_\theta) - C_\theta$$

where I_θ = total energy measured at angle θ, extrapolated to zero time, C_θ = correction factor for extraneous reflections; R_θ is the total energy reflected from all surfaces minus the effect of changes caused by the irradiation. This quantity is measured by extrapolating the temperature curve of the skin back to $t = o$ as shown in figure 3. The time required by the radiometer to reach 94 per cent of its final deflection is 6 seconds (silver reflection curve), and changes occurring after this time are due to heating or cooling of the surface by radiation. As figure 3 shows, the $MgCO_3$ is heated much more by the irradiation than is the skin. The reflecting power of the skin is so small that the temperature change in the skin caused by 7 seconds' irradiation amounts to about 20 per cent to 30 per cent of R_θ. The amount of the skin temperature change caused by the irradiation can be checked by observing the skin temperature for a period after the radiation has been shut off. The result by either method was the same. The manner in which the extrapolation was carried out is shown in figure 3 for silver on glass, skin, and $MgCO_3$. The heating curve is so regular that no appreciable error is made by extrapolating the time six seconds. There is no measurable temperature change in the silver surface during even thirty seconds of exposure.

With this procedure the amount of light reflected from the surface at various angles from the normal can be measured. The silver surface and the $MgCO_3$ were plane surfaces whereas the forehead is irregularly curved. To make sure that this method would apply to the forehead a control experiment with irregularly shaped $MgCO_3$ was made. The total reflection from the $MgCO_3$ was the same whether plane or irregular, although the angular distribution of the reflected light was different in the two cases.

It was found that the angular distribution of reflection followed Angstrom's law of scattering so closely as to allow its use in computing the total reflection. In figure 4 are shown scattering curves for the silver surface, the smooth $MgCO_3$, and the skin. Integrating these curves over the hemisphere gives the values D and B from which the reflection can be calculated from the following equation:

$$\text{Per cent of light reflected} = \frac{Q_0 D^2 \ln u^2}{B(u^2 - 1)} \frac{1}{A.F} \times 100$$

where $Qo = R_o$ = deflection at $\theta = o$; D = distance from aperture to radiometer; B = deflection produced by direct beam; u = constant; A = area of aperture; F = obliquity factor.

The obliquity factor, F, can be computed and also measured experimentally by assuming 98 per cent as the reflecting power of silver. The experimental value was 0.68 and the computed value 0.73; the experimental value was used in the calculations.

The values for the reflecting power of skin and $MgCO_3$ are shown in table 1.

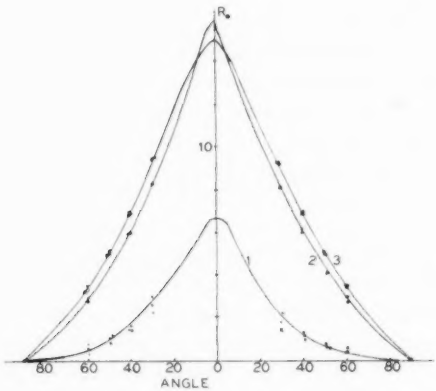


Fig. 4

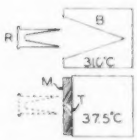


Fig. 5

Fig. 4. Distribution of reflected energy with angle. Curve *1*, skin × 0.1. Curve *2*, $MgCO_3$ × 0.1. Curve *3*, silver × 1.0.

Fig. 5. Arrangement for measuring emissive power of beef muscle and beef fat.

Coblentz (8), using restrahlen, found the reflection of $MgCO_3$ at 4μ as 10.8 per cent and at 24μ as 8.8 per cent. The values given in table 1 are in agreement with the work of Coblentz. The average value for the reflection of skin as computed from the previous work of Hardy and Muschenheim for the radiation from the hot stove is 5.4 per cent compared to 4.6 per cent as measured here. Thus the agreement for reflection from the skin for the separate methods is excellent. The reflection of CO_2 averages 1.3 per cent and is the value of interest in the measurement of the emissivity. From this value of the reflection, E = 98.7 per cent ± 0.5 per cent.

The above results confirm the past observations on the black body character of the skin and are in agreement with all past work on the

reflecting power of skin and $MgCO_3$. They do not agree with the last portion of Büttner's reflection curve.

II. *The emissivity of tissue.* Since 1922 attempts have been made to apply the Stefan-Boltzmann law to the human body. Aldrich obtained evidence indicating that the skin radiated more perfectly than a theoretical black body. Aldrich interpreted his results as evidence of radiation from the deeper layers of the skin. Cobet and Bramigk, in an excellent study, found the skin perfectly black in the far infra-red. In a study of the matter (1932) I confirmed the observations of Cobet and Bramigk and offered an explanation of the anomalous results of Aldrich. Using a technique much like Aldrich, Christiansen and Larsen came to the surprising conclusion that the emissivity of the skin could change from 0.84 to 1.0 depending on the amount of blood in the underlying tissue. In

TABLE 1

Diffuse reflectivity in the infra-red region for silver, $MgCO_3$, and skin

	1.3 μ-50 μ STOVE RADIATION	3.5 μ-50 μ CO_2 RADIATION
Silver	98%	98%
$MgCO_3$	11%	6.1%
	10	8.6
	10	
Skin	4.9%	1.7%
	3.3	2.2
	5.0	1.1
	5.0	1.8
	5.0	0.9

all of these studies it was assumed that the skin temperature could be measured precisely by methods not depending upon the radiation. It is certain that in none of these cases was the temperature of the skin accurately known save in the experiments of Cobet and those in which I followed Cobet's methods. However, even in these latter studies the average surface temperature was not known with sufficient precision. In 1937, Büttner devised the simple and clever method of determining the skin temperature by heating up a radiometer until the energy exchange between it and the skin was zero. In this circumstance the radiometer would have to be at the skin temperature.

As the emissivity of a surface depends upon its composition and texture, it seemed that some light could be thrown on this subject by measuring the constant for muscle tissue and fat tissue. For this purpose smooth

slices of beef muscle and beef fat, which could be obtained in large and thermally homogeneous masses, were used.

A piece of fresh beef muscle 10 cm. square and 1.5 cm. thick was sewed through at different depths with fine wire thermocouples. The depths of the thermocouples were measured at the end of the experiment by cutting through the middle of the piece. The tissue with the thermocouples inserted was pasted to the side of a Leslie cube, the water of which was kept constant at about 37.50°C. ± 0.01°C. The surface of the tissue soon became dried and at the end of an hour thermal equilibrium was established. The temperature of the surface was measured by extrapolating to the surface the thermal gradient obtained from the thermocouple measurements. This is shown in figure 5. The temperatures were measured to 0.01°C. and the depths of the thermocouples to 0.01 cm. so that the gradient was accurately determined. Extrapolation gives the actual temperature of the surface, T_s.

A black body, B, of known temperature, T_b, was mounted to one side of the tissue surface, S, so that the radiometer, R, could move from a position facing B to a position facing S. The deflections in the two positions were measured.

The temperature of the radiometer, T_r, may be considered the same in the two cases as it could not possibly change more than 0.02°C. The difference in the two deflections is

$$D_s - D_b = K(E_s k(T_s^4 - T_b^4) + (1 - E_s)k(T_r^4 - T_b^4))$$

In this equation all the quantities are known except E_s which is thus determined. The second term of the equation is almost zero if E_s be near unity and T_r very near to T_b. T_r may be accurately measured by adjusting T_b so that $D_b = O$, in which case $T_r = T_b$. Numerical example:

Thermocouple no. 1	= 31.57°C.	0.5 mm.	K = 5.45
2	= 31.84	1.0	D_s = 40.8
3	= 34.03	5.0	D_r = 34.0
4	= 35.75	8.0	T_b = 30.53°C.
5	= 37.08	10.0	T_r = 26.54
6	= 38.72	14.0	
Extrapolated surface	31.29	0.0	

Thus,

$$\frac{40.8 - 34.0}{5.45} = E_s \times 1.25 + (E_s - 1)\, 5.92$$

$$E_s = \frac{7.17}{7.17} = 1.00$$

In table 2 are given values for six experiments on beef muscle and five experiments on fat. These values are in approximate agreement with the values found for reflection of cold radiation. Taking an average of the

values obtained by the two methods gives $E_s = 98.9$ per cent $\pm$ 1 per cent as the best value for muscle, fat and also for skin. This value does not depend on blood flow or skin temperature; it depends solely on the character and composition of the skin surface and is a constant for the spectral region beyond 4μ. The low values of Christiansen and Larsen are not confirmed by this work.

TABLE 2

Emissivity for substances estimated from surface temperature extrapolations

BEEF MUSCLE	BEEF FAT	RUBBER
100.2	99.5	98.9
98.2	98.6	98.1
99.7	98.5	
100.1	99.2	
99.7	100.5	
98.4		
Average 99.3	99.3	98.5

Discussion. In order that any experiment on emissivity be valid it is necessary to have a dependable experimental black body. Christiansen and Larsen and later Büttner criticized my work because of the type of black body used. In order to meet this criticism I had constructed a black body similar to that of Christiansen and Larsen. The only difference lay in the fact that the cone of my cube was horn shaped so as to prevent reflection from the tip of the cone. This black body I compared carefully with the one previously used and the two were found to be the same within 0.1 per cent. This confirms further my former check made with a radiation standard of the U. S. Bureau of Standards.

As regards the findings of Christiansen and Larsen it can be said that such results are to be expected when skin thermocouples are used in a hot air bath. At high temperatures one would expect low values of E just as, at low temperatures, Aldrich found high values when he measured skin temperature with a fine-wire thermocouple pressed against the skin. Precise measurements of skin temperature are extremely difficult to make with contact thermocouples. Further, the emissivity of the skin at 9μ is almost certainly not affected by color changes at 0.6μ as suggested by these authors.

The work of Büttner was cleverly and carefully done but no correction was made for the heating of the skin by the warm radiometer. If the warm skin (33.5°) which was previously exposed to room surroundings at 20°C. were suddenly exposed to a warm radiometer (33.5°C.) its temperature would rise 0.25°C. in 15 seconds. This would be due to the gain

of 0.002 cal./sec./cm.^{2} in radiation of the skin from the warm radiometer. The time of response of most thermopiles is usually as long or longer than this. Büttner in a private communication states that the time was from 5 to 10 seconds. Thus, one could assume that the skin temperature would rise about 0.13°C. at least. The difference in the emissivity values found by Büttner and myself is 3.5 per cent. If the cool black body in his experiments were 10°C. below skin temperature this rise in skin temperature would account for two-thirds of the difference. This would bring our values into approximate agreement; that is, within 1.5 per cent which would be within the range of overlapping experimental error.

Büttner, in calculating the contribution to the radiation by deeper layers of skin, unfortunately used the transmission curve for desiccated skin as reported by Hardy and Muschenheim. If the value for wet or normal skin is used, the transmission through 0.03 mm. is about 3 per cent to 4 per cent and the skin may be considered as practically opaque to radiation beyond 5μ. As the blood vessels of the skin are at least 0.3 mm. or more below the skin surface, no radiation from the blood stream can reach the radiometer, and the color of the skin could in no way affect E. Büttner's calculations support this finding.

CONCLUSIONS

The effective emissivity of the skin has been measured in two ways, by reflection and by the Stefan-Boltzman law. The values lie between 98 per cent and 99 per cent with an average of 98.9 per cent $\pm$ 1 per cent. This value agrees with the value obtained by Büttner when certain corrections are made, and with observations of other authors.

REFERENCES

(1) Christiansen, S. and T. Larsen. Skandinav. Arch. f. Physiol. **72:** 11, 1935.
(2) Büttner, K. Strahlentherapie **58:** 23, 1937.
(3) Saidmann, J. Compt. Red. Acad. Science, Paris **197:** 1204, 1933.
(4) Hardy, J. D. J. Clin. Investigation **13:** 593, 1934.
(5) Hardy, J. D. and C. Muschenheim. J. Clin. Investigation **13:** 817; **15:** 1, 1936.
(6) Aldrich, L. B. Smithsonian Misc. Collections, no. 6, p. 81, 1928.
(7) Cobet, R. and Bramigk. Deutsch. Arch. f. Klin. Med. **144:** 45, 1924.
(8) Coblentz, W. Carnegie Inst. of Washington, Pub. no. 35, 1905.

INFLUENCE OF THE HYPOPHYSIS ON CARBOHYDRATE METABOLISM[1]

SAMUEL SOSKIN, R. LEVINE AND W. LEHMANN

From the Department of Metabolism and Endocrinology, Michael Reese Hospital,[2] and the Department of Physiology, University of Chicago, Chicago, Illinois

Received for publication June 23, 1939

The function of the pituitary gland in respect to carbohydrate metabolism can be explained in one or both of two ways. Either the hypophysis stimulates sugar production by the liver, in which case the effects of hypophysectomy should be ascribed to a decreased gluconeogenesis while pituitary extracts accelerate this process; or the hypophysis inhibits the use of sugar by the extra-hepatic tissues, in which case it must be supposed that hypophysectomy accelerates and pituitary extracts depress sugar utilization. Both of these views have been put forward by various authors.

The decreased nitrogen excretion following removal of the pituitary gland, and the low D:N ratios exhibited by hypophysectomized-depancreatized animals led Houssay (1) (2), Long and others (3) (4) to conclude that hypophysectomy decreases gluconeogenesis from protein. Soskin et al. (5) showed that meat-fed hypophysectomized dogs had no difficulty in sustaining their blood sugar levels at the expense of the amino-acids derived from the gastro-intestinal tract. But when such animals were fasted, they were unable to catabolize their own body proteins to amino-acids rapidly enough to support a gluconeogenesis sufficient to maintain their blood sugar levels (6). Since orally administered fat did not maintain the blood sugar level of fasting hypophysectomized dogs (5), it was concluded that such animals suffered from a disability to derive an adequate blood sugar supply from both protein and fatty acid, while fed hypophysectomized animals were limited to ingested protein as their chief non-carbohydrate source of blood sugar. All this evidence favors the view that the hypophysis affects carbohydrate metabolism by influencing the rate of gluconeogenesis.

On the other hand, Fisher, Russell and Cori and others concluded that the effects of hypophysectomy resulted from a relatively greater oxidation of carbohydrate (7) (8) (9) and that the administration of pituitary extracts inhibits the utilization of carbohydrate by fixing the body glycogen (the so-called "glycostatic effect") (9) (10) (11). Their conclusions were

[1] Presented before the American Physiological Society, April, 1939.

[2] Aided by the Max Pam Fund for Metabolic Research.

based upon the calculation of the amounts of carbohydrate oxidized from R. Q. estimations, and upon carbohydrate balance experiments performed on *intact* fasting animals. Aside from the fact that it is becoming increasingly more difficult to accept quantitative deductions concerning the oxidation of foodstuffs based on R. Q. determinations in the whole animal (12), other workers have reported conflicting respiratory quotients (2) (13). We thought it important, therefore, to obtain direct data on the rate of sugar consumption of the extra-hepatic tissues in hypophysectomized dogs, and in normal dogs treated with active anterior pituitary extracts. All these animals were studied after abdominal evisceration in order to eliminate the variable factor of hepatic gluconeogenesis (which invalidates balance experiments on the intact animal) by substituting for it a constant injection of known amounts of dextrose.

METHODS. Both the normal and the hypophysectomized dogs which we used for the carbohydrate balance experiments, were fasted for three days and abdominally eviscerated on the morning of the fourth. Dextrose was given by constant injection, in amounts depending upon the blood sugar level it was desired to maintain throughout a particular experiment. The chemical methods and calculations employed for the carbohydrate balance have been given in detail in our previous publication on sugar utilization by normal and depancreatized dogs (14).

The hypophysectomized dogs had been operated upon from 6 to 10 weeks before being used for the present experiments, and the completeness of the removal of the hypophysis had been checked by the hypoglycemic effect of fasting, and was re-checked by autopsy at the end of the present experiments.

The normal animals were treated with anterior pituitary extract for 3 days preceding the day of evisceration. The extract used was "Phyone",[3] and doses of 2 cc. per dog per day intramuscularly were used.

RESULTS. Before undertaking the carbohydrate balance experiments it was necessary to determine whether the anterior pituitary extract we proposed to use produced effects similar to those described for the various preparations used by other workers. Accordingly Phyone was administered to fasting hypophysectomized dogs, and to hypophysectomized rats. It may be seen from figure 1 that Phyone, in the doses we used, maintained the blood sugar level of fasting hypophysectomized dogs, in sharp contrast to the rapid development of hypoglycemia when these same animals were not treated. The data in table 1 clearly show that Phyone-treated hypophysectomized rats exhibited greater glycogen stores after fasting, than did the control group of untreated fasting hypo-

[3] An extract of the anterior lobe of the pituitary gland, prepared and assayed according to the method of Van Dyke and Wallen-Lawrence, and supplied by Wilson & Company, through the courtesy of Dr. David Klein.

physectomized rats. Thus Phyone resembles the pituitary extracts used by Russel, in producing what she has named the "glycostatic effect" (9).

Was the prevention of hypoglycemia and the increase in glycogen stores, which resulted from Phyone administration, due to an inhibition of carbohydrate utilization and a fixation of the glycogen stores? The carbohydrate balance experiments summarized in figure 2 show that Phyone caused no decrease in the rate of sugar utilization of normal animals at any blood sugar level, when compared with normal untreated animals. The data in table 2 also indicate that the Phyone-treated animals did not differ from the untreated normals in the stability of their glycogen stores, as judged by the rate of decrease of muscle glycogen and the rate of increase of the blood lactic acid during these experiments. It is evident that the effects of pituitary extract are not due to an inhibition of carbohydrate utilization or to a "glycostatic effect."

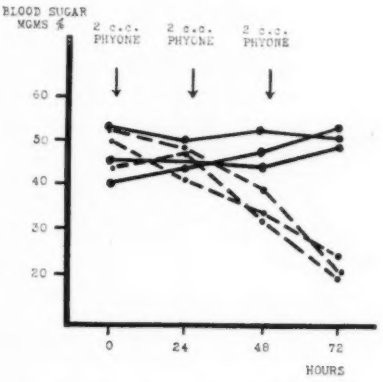


Fig. 1. The sustaining effect of anterior pituitary extract (Phyone) on the blood sugar level of fasting, hypophysectomized dogs. The broken lines indicate the development of hypoglycemia in 3 different animals during the control fasting period. The animals were then fed for a number of days. The continuous lines represent the maintenance of the blood sugar level when the same 3 animals were again fasted, but given Phyone as indicated.

TABLE 1

HYPOPHYSECTOMIZED RATS FASTED 9 HOURS		HYPOPHYSECTOMIZED RATS FASTED 9 HOURS, BUT GIVEN 1 CC. PHYONE PER DAY FOR 2 DAYS PRIOR TO FAST	
Liver glycogen	Muscle glycogen	Liver glycogen	Muscle glycogen
mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
90	170	2,160	230
10	20	90	160
0	40	810	280
720	410	730	320
40	180	1,130	400
20	250	3,470	680
		2,830	620
		40	230
		210	270
		500	490
Average 130.0	178.3	1,197.0	368.0

Was the hypoglycemia of our fasting untreated hypophysectomized dogs due to an increased rate of utilization of carbohydrate, and a decreased stability of the glycogen stores? Figure 2 clearly shows that the extra-hepatic tissues

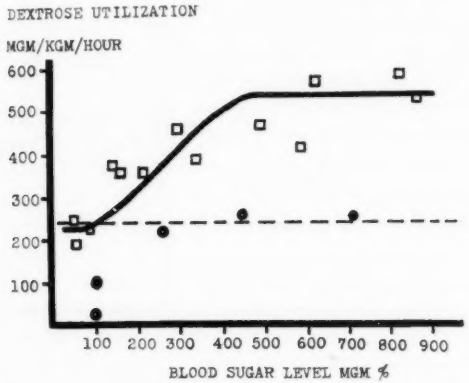


Fig. 2. The influence of hypophysectomy and of the administration of anterior pituitary extract (Phyone) on the rates of sugar utilization of abdominally eviscerated dogs, maintained at various blood sugar levels by the constant injection of the appropriate amounts of dextrose. Each point on the graph represents a complete experiment on a different animal. The round dots represent hypophysectomized dogs; the hollow squares represent normal dogs given Phyone. The smooth continuous curve which is included for comparison, is derived from previous work (14). It indicates the utilization of sugar by untreated, normal eviscerated dogs. The broken horizontal line marks the rate of sugar utilization of normal dogs at their normal glycemic level.

TABLE 2

CONDITION	NUMBER OF DOGS	AVERAGE INITIAL MUSCLE GLYCOGEN	AVERAGE FINAL MUSCLE GLYCOGEN	AVERAGE INITIAL BLOOD LACTIC ACID	AVERAGE FINAL BLOOD LACTIC ACID	AVERAGE DECREASE IN MUSCLE GLYCOGEN	AVERAGE INCREASE IN BLOOD LACTIC ACID
		mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent/ hour*	*mgm. per cent/ hour*
Normal	15	511	355	50.5	106.7	43.1	15.2
Normal, given anterior pituitary extract	13	601	448	59.0	124.6	42.5	18.2
Depancreatized	12	337	217	118.1	183.2	38.4	21.0
Hypophysectomized	5	584	570	27.3	62.8	4.1	9.5

of hypophysectomized animals utilize less than the normal amounts of sugar at equivalent blood sugar levels. The data in table 2 also show that (in contrast to normal untreated, Phyone-treated, and depancreatized animals) the hypophysectomized dogs exhibited practically no fall in

muscle glycogen and a relatively minimal rise in blood lactic acid during the experimental periods. It is obvious that hypophysectomy does not accelerate the use of carbohydrate or mobilize glycogen stores. As a matter of fact, the term "glycostatic effect" could more appropriately be applied to the phenomena observed in the hypophysectomized animals, than to the effects which follow the administration of pituitary extract.

Discussion. Our results show that anterior pituitary extracts do not inhibit the utilization of sugar nor does hypophysectomy accelerate this process. On the contrary, the removal of the hypophysis leads to a diminished rate of sugar utilization at any blood sugar level. This evidence is incompatible with the belief that the function of the pituitary gland in respect to carbohydrate metabolism is to inhibit the use of sugar. The alternative explanation of pituitary function must therefore be adopted, namely, that this gland stimulates sugar production by the liver. This is in agreement with the earlier work of others (2) (15) and ourselves (5) (6), and has been more directly confirmed by Crandall and Cherry (16) since the present work was completed. The latter authors observed the blood sugar contents of the inflowing and outflowing hepatic blood, in intact non-anesthetized normal and hypophysectomized animals, by means of the London cannula technique. They estimated that the rate of sugar output from the livers of their fasting hypophysectomized dogs was only about 50 per cent of the output from the livers of fasting normal dogs. It follows, therefore, that the hypoglycemia of fasting in hypophysectomized animals must be due to a diminished rate of hepatic sugar formation, which is insufficient to meet even the decreased amounts of carbohydrate being utilized by the extra-hepatic tissues.

It is evident, also, that the term "glycostatic effect," which has been applied to the decreased depletion of glycogen stores when pituitary extracts are administered to fasting animals, is a misnomer. This erroneous conception ignores a fundamental axiom in carbohydrate metabolism, namely, that the carbohydrate content of an animal at any time is the resultant of a dynamic balance between the rates of carbohydrate formation and utilization. With this in mind, it will readily be appreciated that the loss of body carbohydrates by an *intact* fasting animal over a period of time, cannot be accepted as an index of the amount of carbohydrate utilized during that period, since this ignores the unknown amounts of sugar formed by the liver during the same interval. From our present results it is clear that the greater loss of total body carbohydrates observed by Fisher, Russell and Cori (8) in fasting hypophysectomized rats as compared to fasting normal controls, was due to a depression of hepatic gluconeogenesis in the former animals, to such an extent that even the low rate of sugar utilization depleted the available peripheral glycogen stores. It is also apparent that the increase in glyco-

gen storage which resulted from pituitary extract administration to their fasting hypophysectomized animals (10) (11) was due to an increased gluconeogenesis, and not to a "glycostatic effect."

The above considerations also apply to the various other phenomena which have been mentioned as resulting from hypophysectomy or from the administration of pituitary extracts. The extreme sensitivity of the hypophysectomized animal to insulin is partly due to the inability of the liver to respond to hypoglycemia with adequate blood sugar formation. This also explains the low sugar excretion following phlorhizin administration to such animals. The amelioration of pancreatic diabetes by hypophysectomy cannot be explained on the basis of greater sugar utilization by the extra-hepatic tissues, for we have shown that the removal of the hypophysis decreases the rates at which sugar is used. Indeed, a comparison of our present with our previous results (14) shows that hypophysectomy decreases sugar utilization below that of depancreatized animals. This is supported by the work of Reid (17) who showed that hypophysectomized-depancreatized cats given intravenous injections of sugar used less of the administered carbohydrate than did cats with only the pancreas removed.

CONCLUSIONS

1. An anterior pituitary extract (Phyone) which was shown to be capable of maintaining the carbohydrate levels of fasting, hypophysectomized animals, was studied for its effect on the utilization of carbohydrate.

2. The extract did not influence the rate of sugar utilization by the extra-hepatic tissues of normal dogs, as determined by direct balance experiments after abdominal evisceration.

3. The extra-hepatic tissues of hypophysectomized dogs were shown by the same methods to utilize sugar at a considerably lower rate than normal.

4. The decrease in the carbohydrate levels exhibited by fasting hypophysectomized animals is due to a decreased rate of hepatic gluconeogenesis, insufficient to meet even the reduced sugar utilization of the extra-hepatic tissues.

5. Conversely, the increased carbohydrate levels resulting from the administration of anterior pituitary extracts (the so-called "glycostatic effect") is not due to a greater stability of the tissue glycogen, but results from the stimulation of hepatic gluconeogenesis to a rate in excess of the sugar utilization of the extra-hepatic tissues.

6. The reduction of hyperglycemia, glycosuria and ketosis in the depancreatized animal following hypophysectomy, is due to a decrease in the rate of hepatic gluconeogenesis, and not to a greater utilization of sugar by the extra-hepatic tissues.

REFERENCES

(1) Houssay, B. A. and A. Biasotti. Endocrinology **15:** 511, 1931.
(2) Houssay, B. A. New Engl. J. Med. **214:** 961, 1936.
(3) Long, C. N. H. Ann. Int. Med. **9:** 166, 1935.
(4) Long, C. N. H. and F. D. W. Lukens. J. Exper. Med. **63:** 465, 1936.
(5) Soskin, S., I. A. Mirsky, L. Zimmerman and N. Crohn. This Journal **114:** 110, 1935-36.
(6) Soskin, S., R. Levine and R. E. Heller. This Journal **125:** 220, 1939.
(7) Fisher, R. E. and R. I. Pencharz. Proc. Soc. Exper. Biol. and Med. **34:** 106, 1936.
(8) Fisher, R. E., J. A. Russell and C. F. Cori. J. Biol. Chem. **115:** 627, 1936.
(9) Russell, J. A. Physiol. Rev. **18:** 1, 1938.
(10) Russell, J. A. and L. L. Bennett. Proc. Soc. Exper. Biol. and Med. **34:** 406, 1936.
(11) Russell, J. A. This Journal **121:** 755, 1938.
(12) Soskin, S. and I. A. Mirsky. This Journal **114:** 106, 1935.
(13) Lichtman, A. L. J. Biol. Chem. **120:** 35, 1937.
(14) Soskin, S. and R. Levine. This Journal **120:** 761, 1937.
(15) Houssay, B. A., A. Biasotti, E. di Benedetto and C. T. Rietti. Compt. rend. Soc. de biol. **112:** 494, 1933.
(16) Crandall, L. A. and I. S. Cherry. This Journal **125:** 658, 1939.
(17) Reid, C. J. Physiol. **89:** 14P, 1938.

THE EFFECTS OF ACETYLCHOLINE AND ESERINE ON FROG MUSCLE

ROBERT HODES AND S. E. STEIMAN[1]

From the Department of Physiology in the Harvard Medical School

Received for publication June 30, 1939

Since Dale and Feldberg (1934) suggested that acetylcholine might be the transmitter of nervous impulses at the neuromuscular junction, numerous workers have brought forward supporting evidence. Brown, Dale and Feldberg (1936) showed that in normal mammalian muscle, close arterial injection of acetylcholine could elicit a quick shortening, accompanied by an outburst of action potentials (Brown, 1937a), thus indicating a true propagated contraction and not a contracture. If the concept that acetylcholine transmits nerve impulses at the motor nerve endings is to be widely applicable, that agent should play a fundamental rôle in neuromuscular systems other than those of the mammal. Most studies in this regard have been made on amphibia. Brown's work (1937b) on normal and denervated frog muscle shows that, as in mammalian muscle, acetylcholine initiates a contraction which is "all or none" and accompanied by typical action potentials.

An obstacle for the theory of acetylcholine transmission, however, is absence of eserine potentiation of the muscular response to single nerve volleys in amphibia. Most workers have been unable to record any potentiation in the isolated preparation or have found it irregular and slight. Kruta (1935) observed no differences before or after eserine. Cowan (1936) also, using prostigmin, reported negative results. Brown (1937b), studying a circulated muscle, recorded no obvious change with small amounts of eserine and a depression with larger doses. Feng (1937), however, was consistently able to secure potentiation to single shocks in the isolated nerve-sartorius preparation.

The present series of experiments was undertaken to study further the conditions existing in the amphibian neuromuscular system.

METHODS. Large bull frogs (R. catesbeiana), weighing 400–900 grams, were used. They were anesthetized by injecting urethane (2 grams per kgm.) into the dorsal lymph sac. The sciatic nerve was exposed, cut high in the thigh, and the peripheral end prepared for stimulation. All branches of the sciatic, tibial and peroneal arteries, except those to the gastroc-

[1] Medical Fellow of the National Research Council.

nemius, were tied off. A short glass capillary cannula, filled with an anticoagulant dye (chlorazol fast pink), was inserted cephalad into the tibial artery. A Mariotte bottle filled with Ringer was attached to the cannula by a rubber tube, which was clamped except when drugs were injected. For injecting drugs, a long hypodermic needle attached to a tuberculin syringe was inserted into the tube near the cannula and directed towards the capillary. After the drug was discharged into the system a momentary release of the clamp forced it in a retrograde direction into the sciatic artery. The normal circulation then carried the drug to the gastrocnemius. Injections of India ink showed that the delivery was quick and that the perfusion was restricted almost entirely to the gastrocnemius.

The leg was immobilized by means of small brads which firmly fixed the tibia. The Achilles tendon was attached to the short arm of a writing lever which pulled against a strong rubber band. The magnification was about 10-fold, and the muscle usually shortened less than 1 cm. The height of the contractions and the tensions developed showed an approximately linear proportionality. With shielded, silver-wire electrodes the nerve was stimulated maximally at various frequencies by a multivibrator circuit.

No particular precautions were taken to regulate the temperature of the preparation. The animal was covered with damp cotton, and the nerve and muscle were frequently moistened with Ringer. The muscles usually remained in good condition throughout the experiment. After several hours of repeated stimulation the only apparent change was a slight decrease in the contractile tension.

The drugs employed were eserine salicylate (Sharp and Dohme), acetylcholine chloride (Merck), atropine sulphate (Merck), and curare (the crude drug). Acetylcholine injections were invariably intra-arterial. Eserine and curare were sometimes injected into the dorsal lymph sac; atropine, when used, was always administered thus. The drugs were freshly prepared in Ringer and injected in 0.1 to 1.0 cc.

Results. I. *Eserine*. That eserine potentiation to single nerve volleys has been difficult to obtain in the frog has already been noted. Feng (1937) used isolated nerve-sartorius preparations for his positive results, and he reports that intra-arterial injection of eserine was without effect. Likewise, Brown (1937b) asserts that eserine (1 to 100γ) perfused through the vessels of the isolated frog's leg, or delivered in the circulating blood, produced no augmentation. We also have found that intra-arterial injection of eserine (0.01γ to 0.6 mgm.) is ineffective.

It was found, however, that injection of eserine (0.5 to 2.0 mgm. per kgm.) into the dorsal lymph sac potentiated the response. Figure 1B shows a 30 per cent augmentation, 15 minutes after eserine, to shocks delivered once every 8 seconds. The effect persisted even after 10 minutes

of continuous stimulation. The potentiation usually varied between 10 and 40 per cent. In an exceptional case, the increment was 85 per cent. In a few experiments no potentiation could be elicited and the failure could not be correlated with the dose of eserine employed.

The results on normal muscle subjected to indirect stimulation at various frequencies confirm those which Cowan (1938) has reported and need not be described in detail here. The striking changes in the response after eserine are exemplified by figures 2 and 3. Figure 2 illustrates features typical of stimulation at 2 per sec. The normal muscle often exhibits the

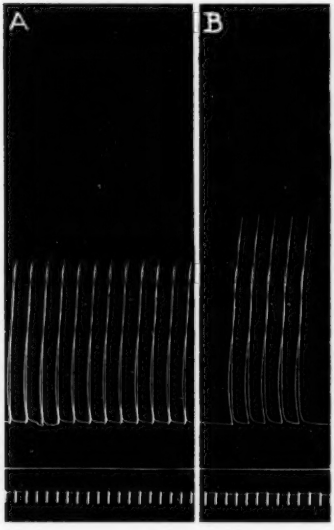


Fig. 1

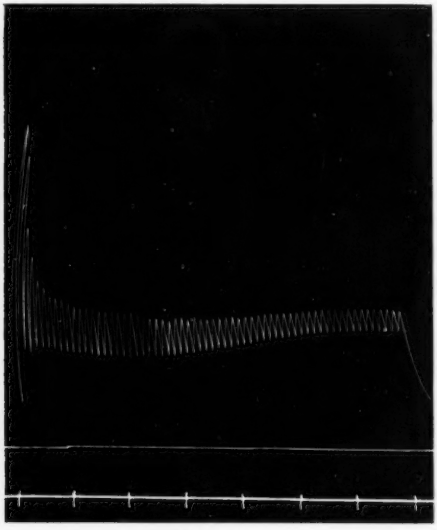


Fig. 2

Fig. 1A. Normal muscle, indirect stimulation 1 per 8 seconds.

B. Fifteen minutes after 1.2 mgm. eserine in lymph sac. In this and all succeeding records, unless otherwise noted, the bottom line records 5-second intervals.

Fig. 2. Stimuli at 2 per second, after 1 mgm. per kgm. eserine in lymph sac.

well-known "treppe" effect for the first few contractions, or maintains a steady tension. After eserine, however, there is a depression evident after the first nerve impulse arrives. In the record the second response showed a 10 per cent decline, and at the fifth shock the muscle responded with a tension which was only 30 per cent of the original. This degree of tension was repeated for 15 to 20 seconds and then with each response a slow rise in tension took place. The general type of record seen here is similar to that which occurs at higher frequencies without eserine (see Cowan, 1938, and cf. following).

Stimulation of a normal muscle through its nerve with slow frequencies (e.g., 9 shocks per sec.) produces a barely complete tetanus (fig. 3A). The maximal tension is maintained for 15 to 20 seconds, after which it slowly declines. After eserine, a smaller initial tension is followed by the Wedensky effect, and later by a gradual rise in tension (fig. 3B). This record resembles that of a normal muscle stimulated at a high frequency (150 per sec.). After a further dose of eserine (fig. 3C) the secondary rise does not take place.

In the normal muscle stimulated indirectly with somewhat higher frequencies (16 to 60 per sec.), the maximal tension is maintained for about 5 seconds, whereupon it slowly falls. After eserine, however, the initial

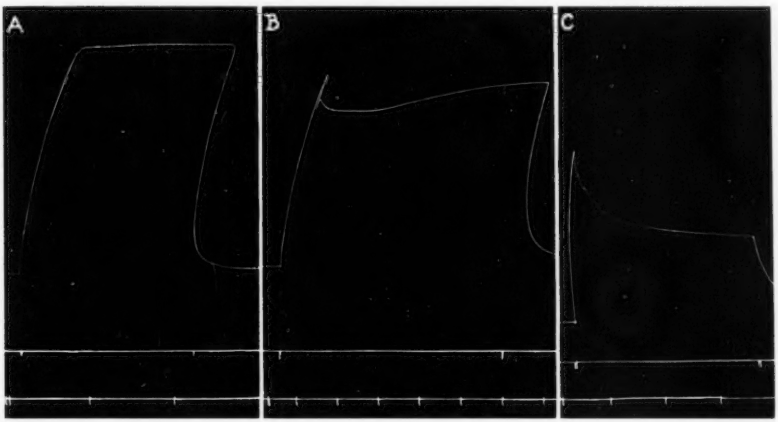


Fig. 3. Stimulation at 9 per second. A. Before eserine. B. After dose of 1 mgm. per kgm. eserine in lymph sac. C. After second dose of eserine, 1 mgm. per kgm.

tension is less than the normal, and is not sustained, but drops rapidly. This fall is succeeded by a slow rise which may exceed the initial tension (cf. Cowan, 1938). If a second dose of eserine is administered to this muscle, a curve similar to figure 3C is produced.

Stimulation at still higher frequencies (100 per sec.) after eserine results in Wedensky inhibition with the tension falling promptly to a low level. The secondary rise may or may not be present. From these results it is evident that the responses obtained by indirect stimulation of normal muscles at high frequencies are reproduced, after eserine, with lower frequencies. Cowan (1938) obtained similar results, not only with eserine, but also with other related substances; and Rosenblueth and Morison (1937) have observed the same effect in the mammal.

II. *Curare.* The maximal response of normal muscles is obtained with

frequencies between 16 and 60 per sec. After curare the responses are lower than normal at all frequencies, the greatest tension appearing with frequencies of 100 to 150 per sec. The secondary rise seen in normal muscles with high frequency stimulation does not take place after curare (cf. Cowan, 1938; Feng, 1938).

Boyd (1932) found that in the curarized cat a partial decurarization resulted from a short tetanus at a high frequency. He was unable to obtain this effect in the frog. That the phenomenon can occur in this animal, however, is illustrated in figure 4. Within limits, the higher the frequency and the longer the duration of the tetanus, the greater the decurarization. If, however, the tetanus is continued for too long a period, no decurarization is obtained; instead, there is a further reduction of the twitches.

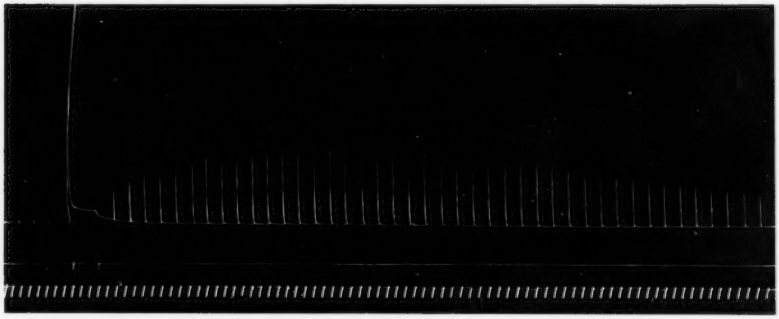


Fig. 4. Curare. Response to single shocks greatly depressed. Between signals, 15-second tetanus at rate of 100 per second, followed by resumption of stimuli at 1 per 8 seconds.

The antagonism between curare and eserine is well known (for references see Rosenblueth, Lindsley and Morison, 1936). Cowan (1938), using repetitive stimulation, has demonstrated this antagonism in frog muscle. We have found that eserine exerts a decurarizing action in a muscle indirectly stimulated once every 8 seconds.

III. *Acetylcholine.* In the fresh muscle small amounts of acetylcholine (1 to 10γ) cause a depression of the muscular response to single shocks delivered to the motor nerve (Brown, 1937b). In a fatigued muscle, on the other hand, we found that acetylcholine frequently results in an increase of the responses.

In the present experiments doses of 5 to 200γ acetylcholine were used. Injection of 10γ caused a slight depression of the response of a muscle indirectly stimulated once every 8 seconds. The depression was transitory and the tension soon returned to its former level. If a second dose of 10γ

acetylcholine was administered, the resulting depression was greater in magnitude and duration than the preceding one. Subsequent similar doses, after recovery of the muscle, elicited more marked depressions than those produced by the earlier injections. The contracture resulting from each successive dose of acetylcholine remained the same.

The depressant action of 50γ acetylcholine is shown in figure 5. After the response returned to normal, 25γ acetylcholine produced a greater and more prolonged depression. Further, administration of 10γ acetylcholine now brought about a greater depression than the two larger previous doses. On the other hand, the contracture elicited by acetylcholine is dependent on the dosage and is not affected by the previous injections of the drug.

If larger amounts of acetylcholine are used, the effect of a previous injection is even more marked. For example, after a muscle had recovered from the effects of 100γ acetylcholine, a second injection of the same

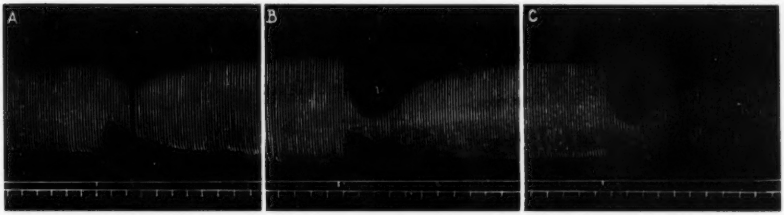


Fig. 5. Atropine, 0.5 mgm. in lymph sac; stimulated 1 per 6 seconds. Time signal, 30 seconds. A. At signal, 50γ acetylcholine. B. At signal, 25γ acetylcholine. C. At signal, 10γ acetylcholine.

quantity of acetylcholine caused an almost complete suppression of the response for about 8 minutes, after which it slowly recovered.

The response of mammalian muscle to acetylcholine given during tetanic stimulation depends on the frequency of stimulation, the dose given, and the period of contraction at which the injection is made (Briscoe, 1936). Rosenblueth and Morison (1937) found that during the intermediate secondary rise produced by high frequency stimulation, acetylcholine produced first a rise in tension, then a fall, and finally another increase. During the fall in tension that follows the secondary rise, the effects of acetylcholine were primarily stimulant. In the present experiments it was found that during the gradual loss of tension which resulted from continuous tetanic stimulation (16 to 100 per sec.), acetylcholine (5 to 50γ) elicited a sharp decline in tension. At higher frequencies of stimulation acetylcholine given during the steady level of contraction caused a sudden marked transient fall of tension (fig. 6). During the period of ultimate decline acetylcholine did not produce a rise in tension. Sometimes a

slight increment was observed but this was not greater than the contracture produced by the same amount of acetylcholine administered without stimulation.

Acetylcholine has a decurarizing effect on mammalian muscle (Briscoe, 1936); (Rosenblueth, Lindsley and Morison, 1936). In the frog, however,

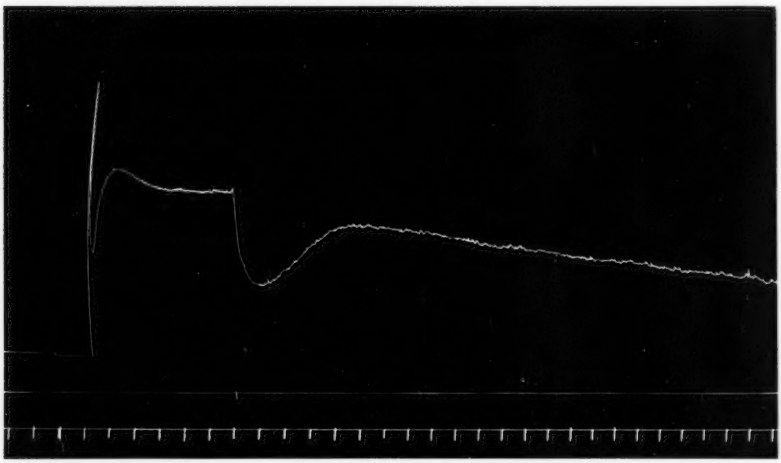


Fig. 6. Normal muscle, stimulated 160 per second. At signal, 30γ acetylcholine

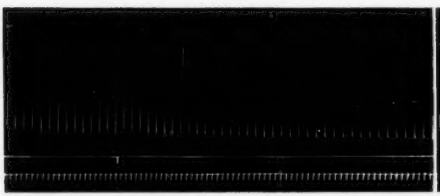


Fig. 7

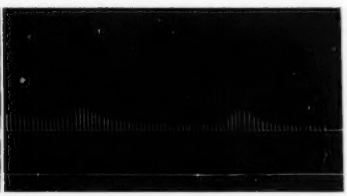


Fig. 8

Fig. 7. Curare. Atropine, 0.5 mgm. Stimulated 1 per 7 seconds. At signal, 30γ acetylcholine.

Fig. 8. Curare. Atropine, 0.5 mgm. Stimulated 1 per 8 seconds. At first signal, 200γ acetylcholine. At second signal, 100γ acetylcholine.

we have repeatedly observed that injection of acetylcholine in partially curarized muscle usually results in a further depression of the response (fig. 7). Brown (1937b) also observed this phenomenon and suggested that his doses of acetylcholine (1 to 2.5γ) were insufficient and that perhaps larger doses would abolish the curare paralysis. In the present study, doses as large as 200γ were usually ineffective in decurarizing the muscle.

In a few cases, however, acetylcholine (10 to 200γ) did have a decurarizing action (fig. 8). The decurarization could not be correlated with the dosage of acetylcholine employed for the different animals. Thus one frog might exhibit decurarization with 10 to 200γ acetylcholine, while another would not show this phenomenon with doses in the same range.

Occasionally acetylcholine, delivered to the partially curarized muscle during stimulation at 1 per 8 sec. would cause an increase in the magnitude of the first response. The decurarization lasted for only a single response, and was followed by a contracture and a depression of the twitches.

Discussion. Feng (1937) and Cowan (1938) have reported the potentiation of single twitches by eserine in the isolated preparation of frog muscle. Both workers pointed out that special conditions were necessary to demonstrate this effect. It has been shown (p. 471) that eserine injected into the dorsal lymph sac will potentiate the response. Relatively large doses of eserine were necessary and even then the effect was not always seen. The failure of Feng (1937) and Brown (1937b) and ourselves to secure potentiation by arterial injection has already been mentioned. Perhaps this failure depends on the speed with which the drug reaches the end plates and the length of time it remains there. Lymph-sac injection would allow a slow transport in small concentrations and it may be that a slow steady influx of the drug at the neuromyal junction is one of the conditions required for typical action.

The effects of eserine on the muscular responses to various frequencies agree closely with the findings in mammalian muscle (see Rosenblueth and Morison, 1937).

If curare is assumed to raise the thresholds for stimulation and paralysis by acetylcholine (Dale, see Briscoe, 1936), the depression of the response after curare would be due to the liberation of subliminal quanta of the mediator. This would explain the shift in the frequencies required to produce the maximal tension following curare (p. 474). The interval between stimuli at the lower frequencies is sufficiently long to permit a destruction of acetylcholine by the esterase to such an extent that the mediator liberated by each nerve impulse is present in an amount subliminal for many fibers. At higher frequencies, however, enough acetylcholine may accumulate so that the concentration reaches the threshold level for some of the inactive fibers.

The decurarizing effect of high frequency stimulation (fig. 4) is similar to that which occurs in the mammal. Since the phenomenon is prolonged (in the frog it often lasts for 15 minutes or longer) Rosenblueth and Morison (1937) suggested that this decurarization is not due to a persistence of acetylcholine but may be caused by a mobilization of potassium ions.

The increasingly greater depressions elicited by successive doses of acetylcholine (p. 474 and fig. 5) indicate that the drug has long-lasting

effects on the neuromuscular system. The rapid destruction of acetylcholine in living tissues is well known. It is difficult, therefore, to explain the cumulative effects as due to the persistence of the drug. The present experiments offer no clue as to the nature of this "sensitization" by previous injections of acetylcholine, nor do they show the site of such action. The phenomenon requires further investigation.

The absence of a stimulant action of acetylcholine in the final decline of tension during rapid repetitive stimulation (p. 475) suggests that the depressant effects of the drug are very prominent in the frog.

The inconsistent effects obtained from acetylcholine after curare indicate that the substance produces not only the decurarization described by Rosenblueth, Lindsley and Morison (1936) and Briscoe (1936), but also some other action not seen in the mammal. Thus acetylcholine may cause two opposed effects after curare: a further depression (fig. 7) or a decurarization (fig. 8). Although a depression was more common, neither this effect nor the decurarization was dependent on dosage (p. 476). The conditions under which the decurarizing action appears could not be defined. It may be that the two opposed phenomena can occur simultaneously and that the depressant activity which usually predominates masks the decurarizing influence of the drug. This view is supported by the atypical effect observed in a few cases in which a dissociation of these apparently independent actions was evident (p. 477).

SUMMARY

1. The effects of eserine, curare and acetylcholine on the normally circulated frog's gastrocnemius subjected to varying frequencies of indirect stimulation were studied.

2. Lymph-sac injections of eserine produce a potentiation of single twitches (fig. 1). Slow frequencies, after eserine, produce responses similar to those seen at higher frequencies in the normal muscle (fig. 3).

3. After curare, the frequency required to produce maximal tension increases (p. 474). The antagonism between curare and eserine is readily demonstrated (p. 474).

4. Short tetani at various frequencies result in a temporary decurarization (fig. 4).

5. After a depressant arterial injection of acetylcholine, subsequent injections have greater effects (fig. 5 and p. 475).

6. Acetylcholine, after curare, may have either a depressant (fig. 7) or a decurarizing action (fig. 8) or, in unusual cases, a combination of both effects (p. 477).

We wish to thank Dr. A. Rosenblueth for his helpful advice during the course of this study.

REFERENCES

BOYD, T. E. This Journal **100:** 569, 1932.
BRISCOE, G. The Lancet **1:** 469, 1936.
J. Physiol. **87:** 425, 1936.
BROWN, G. L. Ibid. **89:** 220, 1937a.
Ibid. **89:** 438, 1937b.
BROWN, G. L., H. H. DALE AND W. FELDBERG. Ibid. **87:** 394, 1936.
COWAN, S. L. Ibid. **86:** 61P, 1936.
Ibid. **93:** 215, 1938.
DALE, H. H. AND W. FELDBERG. Ibid. **81:** 39P, 1934.
FENG, T. P. Chinese J. Physiol. **12:** 51, 1937.
Ibid. **13:** 141, 1938.
KRUTA, V. Arch. Internat. Physiol. **41:** 187, 1935.
ROSENBLUETH, A., D. B. LINDSLEY AND R. MORISON. This Journal **115:** 53, 1936.
ROSENBLUETH, A. AND R. MORISON. Ibid. **119:** 236, 1937.

MOTILITY OF SCHOOL CHILDREN DURING SLEEP

GLENVILLE GIDDINGS

From the Department of Medicine, Emory University, Emory University, Georgia

Received for publication July 17, 1939

The time spent in bodily movement during sleep by normal, healthy adults has been shown by Kleitman, Cooperman and Mullin (1933) to be about half a minute per hour. Renshaw, Miller and Marquis (1933) made an extensive study of the sleep motility of children, but the method used did not afford a true measure of the time spent in movement. The nine hour period in which the children were in bed was divided into one minute intervals; each interval in which the subject moved was recorded as an active minute even though the movement lasted only a fraction of a minute. This method gave an average of approximately 70 active minutes for the nine hour period in bed. The present author (1934, 1936) studied the effects of hot and cold baths, heavy meals, emotional disturbances etc. on children's sleep with the same apparatus and on the control nights obtained the same results as Renshaw. While this method gives characteristic and reproducible "sleep patterns" for individual children and has been found to be reliable for determining the comparative effects of various experimental conditions on sleep, it obviously does not afford a means of measuring the exact time spent in movement. The actual duration of the motility of children during sleep has, therefore, not been determined. A different type of apparatus has been devised for this purpose and has been used in the present investigation.[1]

METHOD. The essential principle of the apparatus, which will be referred to as the chronomotometer, was the electrical control of a counter by vacuum tubes in such manner that the counter advanced one unit each second the bed spring was in motion. In order to make a direct comparison with results obtained with the Renshaw apparatus, to be designated hereafter as the hypnograph, the two methods were employed simultaneously.

The hypnograph operated as follows: A "hypnograph unit" which consisted of a movable bakelite shaft and a stationary frame provided with brush contacts was attached to the bed springs. When the subject moved in bed it made an electrical contact which energized an electro-magnet

[1] The author is indebted to Mr. Philip Stout, electrical engineer, for devising and constructing the apparatus.

in a recording room separate from the dormitory. When the electromagnet was energized, it caused a pen inscribing a base line on a moving kymograph paper to make an offset in the base line. Perpendicular dotted lines were inscribed on the kymograph paper at minute intervals for determining the active minutes throughout the night.

The chronomotometer used the voltage developed across the pen solenoid of the hypnograph. A circuit of the unit of this instrument is shown in figure 1. Since the voltage from the solenoid served merely to change the bias on the grid, there was no interference with the control of the writing pen by the solenoid. When the subject moved, disturbing the bed attachment, pulses were made to flow to the transformer AT-1. These pulses were amplified by the grid of the 885 thyratron tube which in turn immediately lost control, thereby operating the magnetic counter one second. The power to operate the counter came from the condenser C_2

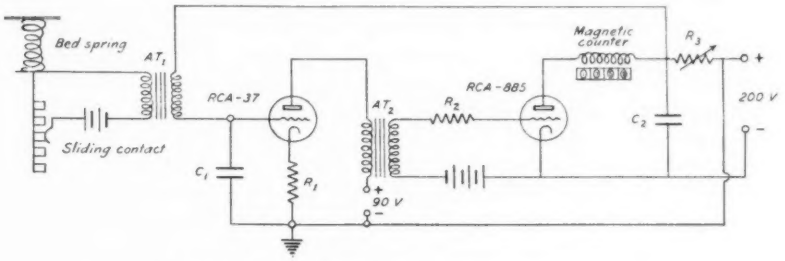


Fig. 1. Schema of a unit of the chronomotometer

which was slowly recharged through the resistor R_3. The system was set by the adjustment of R_3 for one second. When the magnetic counter operated, the voltage across the 885 tube swung negative, due to the magnetic fly wheel action. This allowed the tube to gain control. The buffer tube 37 was made inoperative while C_2 was recharging, thus preventing disturbance to the circuit by additional pulses before the end of the cycle. The accuracy of the recording devices was checked against a stop watch with continuous agitation of the bed. The error was less than one second in two thousand.

Twenty-four units were constructed and built in a rack that stood approximately 6 feet high which required a floor space of 20 by 12 inches. With this arrangement the sleep motility of 24 subjects could be obtained simultaneously. The chronomotometer and polygraph of the Renshaw apparatus were placed in a room adjoining the dormitory and were connected with the bedspring by means of cables. The counters of the chronomotometer were read at half-hour intervals by an attendant who was on duty throughout the night. Relay boxes were so arranged in the

circuit that if there was any interference with the circuit the attendant was notified by the lighting of a signal lamp which remained burning until the condition was corrected. The signal was also given if a subject arose from the bed during the night.

The investigation was conducted in a boarding school in the mountains of Georgia. This school was chosen because of the distinct advantages it offered. Located some distance from the railroad and highway, it was free from noise and other disturbances that interfere with sleep. The daily routine of the children was the same except on Sundays. They went to bed promptly at 8:30 and arose in the morning at 5:30. The nature of the experiment was explained to them and their whole-hearted coöperation readily obtained.

Twelve boys and twelve girls, between the ages of 9 and 14 years, served as subjects of the experiments. Each child was given a physical examination and was found to have no organic defects. Foci of infection discovered on the examination were removed before the experiment was begun. Urinary analysis, a complete blood count, electrocardiographic tracings and blood pressure and basal metabolic rate determinations were made on all the children and found to be normal. A special laboratory was constructed consisting of two dormitories, lavatories and several anterooms for purposes incidental to the experiment. In one dormitory there were twelve beds for the boys and in the other an equal number of beds for the girls. All the springs, mattresses, pillows and bed clothing were identical. The experiment was conducted in the months of April and May.

Results. The results are presented as averages of 14 nights on each subject, or a total of 168 nights for the boys and the same number of nights for the girls. The average number of active minutes for the boys and girls as recorded by the hypnograph is shown from hour to hour for the entire night in figure 2; the total number of active seconds recorded by the chronomotometer in figure 3. Whereas the hypnograph recorded 67.5 and 69.8 active minutes for the boys and girls respectively, the chronomotometer showed that the time actually spent in motion was only 250 and 282 seconds or 4.2 and 4.7 minutes. The first half-hour was the most active period of the entire night. The hypnograph recorded an average of 9.6 and 9.7 active minutes for the boys and girls, respectively, during this period and the chronomotometer 49.4 and 52.3 active seconds. During the second half-hour there was a marked decrease in activity to 1.7 and 1.4 active minutes for the boys and girls, respectively, and 5.7 and 8.1 active seconds. From the second hour onward there was a gradual increase in motility throughout the night which usually reached its highest point the last hour. When the actual time spent in motion during the first half hour was deducted from the total number of active seconds, the activity for the 8½ hours for the boys and girls was reduced to 221.9 and 200

active seconds or an average of 26 and 24 active seconds per hour, respectively. From these observations it is apparent that in two respects, namely, in the total time spent in movement and in the gradual increase in activity throughout the night, the motility of these children between the ages 9 and 14 was the same as that of the adults studied by Kleitman and his associates.

The pronounced activity during the first half-hour was doubtless due to some delay in falling asleep. The individual records showed that there was a marked variation in activity in this period not only from subject to subject but also from night to night in the same subject. One child, for

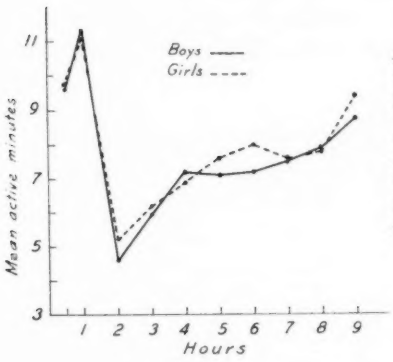


Fig. 2

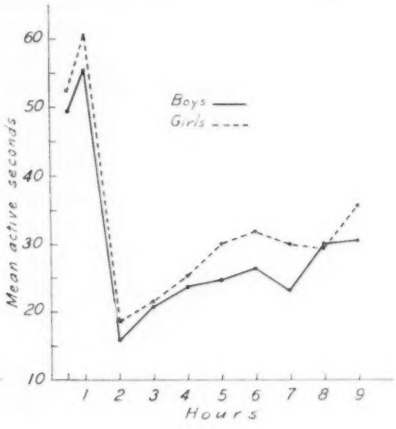


Fig. 3

Fig. 2. Mean active minutes of the second half of the first hour and hourly periods throughout the night (Renshaw apparatus).

Fig. 3. Mean active seconds of the second half of the first hour and hourly periods throughout the night (chronomotometer).

example, showed a variation of 11 to 150 seconds with a standard deviation of $\pm$ 36.1. The coefficient of variation for this period was 70 as compared with a coefficient of 47 and 42 for two other periods taken at random, namely, the 5th and 7th hour. The coefficient of variation of the active seconds of all the girls for the first half-hour was 55 whereas for the entire night minus the first half-hour it was 21. This variation during the first half-hour after retiring is not surprising in view of the common observation that one's mental activities, emotional experiences, etc., before retiring affect the promptness with which one falls asleep. In studying sleep motility it would therefore appear that a more accurate record would be obtained by omitting the first half-hour. In these experiments the standard deviation of the active seconds of the entire night, which was

±65.9 and ±70.4 for the boys and girls, respectively, was reduced to ±55.6 and ±57.3 when the first half-hour was omitted in the calculations.

A sex difference in sleep motility between the ages 10 and 14 was observed by Renshaw using the hypnograph. In the present experiments the chronomotometer showed on a small group no difference between the activity of the boys and the girls. There were 7 boys 11 and 12 years of age and the same number of girls. The average number of active seconds of these two groups from hour to hour throughout the night is shown in table 1.

It will be observed that in each period there was practically no difference in the motility of the two groups. During 8½ hours spent in bed (the first half-hour being omitted) the girls were active only 14 seconds more than the boys. This difference was of no significance as it was only 1.5 times the sigma of the difference.[2] The largest difference in the individual periods occurred in the 7th hour in which the girls were more active an average of 31.2 seconds and the boys 22.5 seconds, or a difference of 8.7 seconds. This difference was likewise of no significance, being 1.6 times its sigma.

TABLE 1

Average number of active seconds during sleep of boys and girls of the same age

SUBJECTS	AGE	HOURS OF NIGHT									TOTAL
		Second half of 1st hour	2	3	4	5	6	7	8	9	
	years										
7 boys	11–12	7.0	17.8	22.9	25.5	26.4	30.3	22.5	33.5	32.3	218.2
7 girls	11–12	5.7	19.1	24.3	25.9	30.7	29.5	31.2	30.6	35.3	232.3

CONCLUSIONS

A new method for studying sleep motility of a number of subjects simultaneously is described.

A study has been made with this instrument of the sleep motility of school children between the ages of 9 and 14 years.

The total length of time spent in motion during nine hours in bed amounted to less than 5 minutes.

The first half-hour was the most active period, doubtless because children ordinarily do not fall asleep immediately upon going to bed.

From the second hour onward there was a gradual increase in motility which reached its highest point in the last hour.

[2] The sigma of the difference was calculated from the formula $\sigma_{diff.} = \sqrt{(\sigma_{M_1})^2 + (\sigma_{M_2})^2}$. The sigma of the mean for both boys and girls was 5.84.

Both as regards the total duration of activity during sleep and the gradual increase in activity throughout the night, the sleep motility of the children in these experiments was the same as that of the adults reported by Kleitman and his associates.

No sex difference was observed in sleep motility. The difference between the activity of boys and girls of the same age amounted to only 14 seconds.

REFERENCES

KLEITMAN, N., N. R. COOPERMAN AND F. J. MULLIN. This Journal **105:** 574, 1933.

RENSHAW, S., V. L. MILLER AND D. P. MARQUIS. Children's sleep. New York, 1933.

GIDDINGS, G. J. A. M. A. **102:** 525, 1934.
Southern Med. J. **27:** 312, 1934.
J. Med. Assn. Georgia **25:** 351, 1936.

THE EFFECTS OF MAGNESIUM UPON DENERVATED MAMMALIAN MUSCLE

CLARENCE A. MAASKE AND BOYCE GIBSON

From the Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago

Received for publication July 10, 1939

The gradual motor paralysis produced by magnesium (Meltzer and Auer, 1905) is primarily due to a block at the junction between nerve and muscle (Lubinska, 1935). Papers from this laboratory (Maaske, Boyd and Brosnan, 1938; Boyd, Brosnan and Maaske, 1938) however, have pointed out certain differences between the junctional effects of curare and of magnesium.

Rosenblueth and Luco (1937) noted that curare, in moderate dosage, depressed the acetylcholine response of denervated mammalian muscle. The same dosage, however, left the twitch response to direct electrical stimulation unimpaired. Magnesium, on the other hand, raises the threshold of normal or denervated frog muscle to direct electrical stimulation (Ashkenaz, 1938). The present report is concerned with the effects of magnesium upon the responses of denervated mammalian muscle to electrical stimuli; and with the effects of magnesium upon the sensitivity of such preparations to acetylcholine and to eserine.

METHODS. Eleven medium sized (about 12 kilo) dogs were used. They were anesthetized with 45 mgm. per kilo nembutal intraperitoneally. A tracheal cannula was inserted, as artificial respiration was necessary when large doses of magnesium were employed. The right tibialis anticus muscle had been denervated, by resection of about one centimeter of the peroneal nerve, 8 to 34 days previous to the experiment. The tendon of the tibialis anticus muscle in each limb was freed and connected to a muscle lever made to pull against a strong rubber band. When necessary a steel spring was interposed between the recording lever and the muscle tendon on the innervated side. The lever excursion varied in approximate linear proportion to the tension developed. The legs were immobilized by drills through the tibia. On the innervated side the sciatic nerve was sectioned high in the thigh and shielded platinum electrodes placed on the peroneal nerve. On the denervated side steel needle electrodes were thrust into the belly and tendon of the muscle. Maximal stimuli, at the rate of one shock every 8 or 10 seconds, were delivered through cored coils activated

by condensers discharging through a thyratron circuit (Schmitt and Schmitt, 1932). Responses of both the normal and the denervated muscle were recorded simultaneously on smoked paper.

All injections were made into the right external jugular vein, which had been cannulated and connected to a burette.

Magnesium was given as a 6.7 per cent solution of $MgSO_4 \cdot 7H_2O$. All other solutions were freshly prepared just previous to their injection.

RESULTS. In all cases the intravenous injection of 20 cc. of a 6.7 per cent solution of $MgSO_4 \cdot 7H_2O$ caused a decrease in the twitch height of both the innervated and denervated tibialis anticus muscle (figs. 1, *6*; 2, *4*; 3, *14*, *2*). This depression reached its maximum within two minutes following the injection. Recovery began immediately on the normal side,

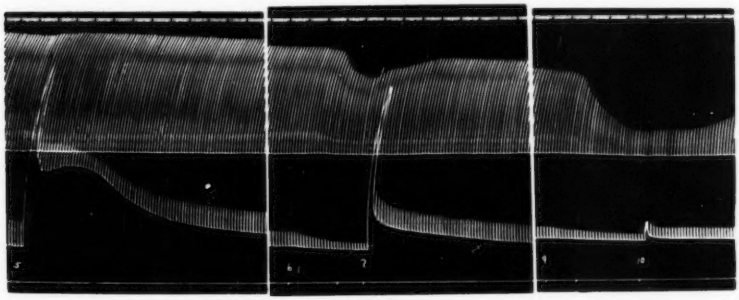


Fig. 1. Male dog, 14 kilo; 14 day denervation. Upper record contractions of tibialis anticus muscle stimulated through peroneal nerve. Lower record responses of denervated tibialis. Time 5 second intervals. *5*, 0.5 mgm. per kilo ACh. intravenously. *6*, 20 cc. $MgSO_4$ (6.7 per cent) intravenously. *7*, 0.5 mgm. per kilo ACh. intravenously. *9*, 20 cc. $MgSO_4$ intravenously. *10*, 0.5 mgm. per kilo ACh. intravenously.

and the original twitch height was restored within a few minutes; but on the denervated side, even after half an hour, there was little or no evidence of recovery.

The prolonged depression of the denervated muscle is not due to polarization nor to local injury at the electrodes. After several injections of magnesium with considerable depression of the denervated muscle and complete neuromuscular block we have tried, without success, to increase the twitch height by shifting the position of the electrodes. In the absence of magnesium, twitch heights remained nearly constant for a period of an hour or more. Such a period was as great as the time during which magnesium effects were recorded.

Each injection of $MgSO_4$ produced a greater (but more transient) depression of the recorded twitch heights of the innervated side. When

neuromuscular block was established it was still possible to produce a further depression of the denervated muscle. With continued administration of magnesium, until the death of the animal from cardiac failure, we

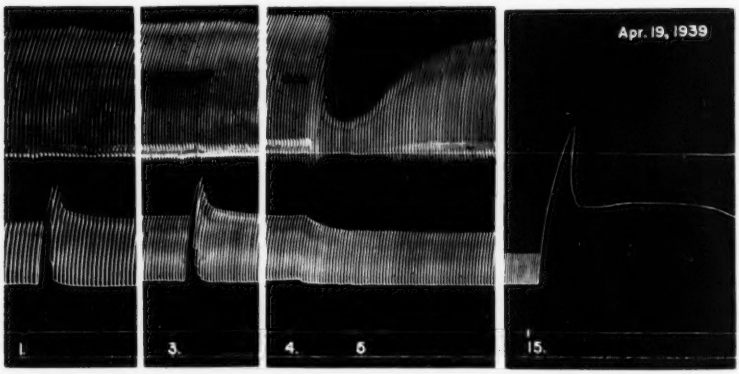


Fig. 2. Dog of 17 kilo; 28 day denervation. Upper record innervated side, lower record denervated tibialis anticus muscle. Time 5 second intervals. All injections intravenous. *1*, 0.5 mgm. per kilo ACh. in 0.9 per cent NaCl. *3*, 0.5 mgm. per kilo ACh. in 6.7 per cent $MgSO_4 \cdot 7H_2O$. *4*, 20 cc. $MgSO_4$. *5*, 0.5 mgm. per kilo ACh. in isotonic saline. *15*, 0.5 mgm. per kilo ACh. (saline) 8 minutes after eserine (0.5 mgm. per kilo) and atropine (1.0 mgm. per kilo).

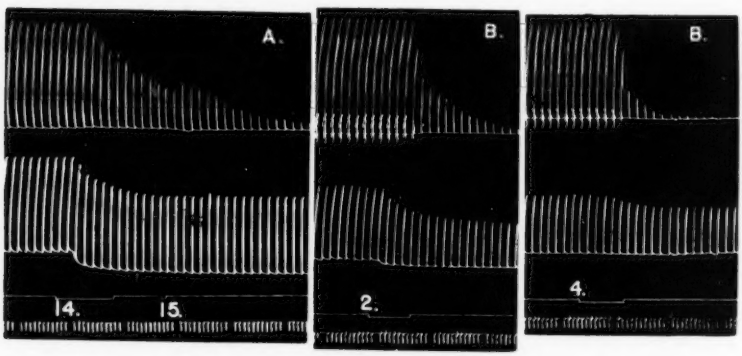


Fig. 3. A, 8.6 kilo dog; 8 day denervation. All injections intravenous. *14*, 20 cc. $MgSO_4$. *15*, 0.5 mgm. per kilo, ACh. in 6.7 per cent $MgSO_4$. B, 8.6 kilo dog; 13 day denervation. *2*, 20 cc. $MgSO_4$. *4*, 20 cc. $MgSO_4$ 30 minutes after no. 2.

were never able completely to suppress the responses of the denervated muscle to electrical stimulation.

Control injections of an equal amount of isotonic saline had no visible

effect upon the twitch heights of either denervated or innervated preparation. Clamping the abdominal aorta for a period greater than the injection time (1 min.) produced no changes of the myograms, nor have we seen an appreciable change in the myogram for a short period after the death of the animal. Likewise insufflation of amyl nitrite (to produce a peripheral vasodilatation) did not alter in any respect the myographic tracings. The drop in blood pressure which magnesium produces cannot be primarily responsible for the depressed twitch height.

As seen especially in figure 3, *14*, there is a coincident drop in the base line of the denervated muscle, which we have never seen on the innervated preparation. This lengthening of the denervated muscle by magnesium is interesting in its implications.

The effect of the magnesium is cumulative and progressive. After apparent complete recovery of the nerve-muscle preparation a second equal dose of $MgSO_4$ is more effective than the first (fig. 1). There is, however, no further drop in the base line of the denervated side. (fig. 3, *2* and *4*).

Responses to acetylcholine following magnesium. Magnesium abolishes the sensitivity of denervated muscle to acetylcholine. After administration of 20 cc. of isotonic $MgSO_4$, the contracture response to a moderate dose of ACh. is either lost (fig. 2, *5*) or much reduced (fig. 1, *10*). In the absence of magnesium, the same dose of ACh., repeated at intervals, evokes uniform contractures.

The effect of magnesium upon the sensitivity of denervated muscle to ACh. is relatively transient. When the twitch height on the innervated side has returned to normal, the contracture of the denervated muscle, in response to ACh. also has returned to normal. This contrasts strikingly with the prolonged depression of the twitch responses to electrical stimulation. And eserine (0.5 mgm. per kilo) restores the response to ACh. in spite of the continued presence of magnesium (fig. 2, *15*). One milligram per kilo atropine sulfate was always given with or just previous to the eserine injection. The figure does not show the return of electrical excitability as the dose of ACh. employed was high and the contracture quite prolonged.

The ineffectiveness of ACh. after magnesium is not due to direct interaction between the two. As seen in figure 2, *1* and *3*, there is no observable difference in the response whether the acetylcholine bromide was dissolved in 0.9 per cent NaCl, or in 6.7 per cent $MgSO_4 \cdot 7H_2O$.

Discussion. The results of these experiments show that magnesium in addition to its previously described actions, raises the threshold of intact denervated skeletal muscle to electrical stimulation. Both magnesium and curare may depress the sensitivity of denervated muscle to ACh.; but magnesium though acting similarly to curare has an additional effect.

The depression of twitch height we have described during magnesium "curarization" of denervated mammalian muscle in situ suggests several possibilities. 1. The threshold of individual fibers has been raised, thus eliminating units remote from the stimulating electrode. Since the stimulation applied to the nerve was somewhat above maximal, it may be presumed that the motor fibers were all excited by each shock. A shock which, when applied directly to denervated muscle, would be maximal for all contractile units would necessarily be much above the threshold for those fibers near the cathode. 2. Individual fibers are no longer contracting maximally; or 3, some fibers, even though stimulated with a maximal shock, have ceased contracting entirely. Ashkenaz (1938) reports an increase in threshold of single muscle fibers (both denervated and innervated) of the frog to electrical stimulation. Although it is somewhat hazardous to transfer to mammalian tissue conclusions drawn from cold blooded animals, the results of Ashkenaz indicate that we are more than likely dealing with a depression of muscle irritability.

Lubinska (1935) noted that although there was a marked increase of muscle chronaxie during magnesium narcosis the heterochronism of muscle and nerve did not necessarily change at the moment neuromuscular block was established, nor at the instant of recovery from the block. Recovery from magnesium neuromuscular block is fairly rapid and complete, whereas the depressant action upon the denervated muscle is more prolonged. Ashkenaz has noted that in the frog, denervated single muscle fibers are depressed longer than the innervated ones. Because of the prolonged direct action of the magnesium upon the muscle cell, caution must be exercised in comparing curare neuromuscular block with the similar but more transient condition produced by magnesium.

Just as in curare poisoning of denervated muscle (Brown, 1937; Rosenblueth and Luco) magnesium raises the threshold of denervated muscle to ACh., but the secondary contracture in this case was abolished first (and apparantly more completely).

Because denervated muscle depressed with magnesium is still irritable to electrical stimulation but loses its sensitivity to ACh. two possibilities are suggested: 1, two different mechanisms of excitation are involved (Blair, 1938); 2, different contractile elements are responding (Knowlton and Hines, 1937). The first possibility seems to carry the weight of evidence particularly because during ACh. contracture of a denervated muscle the excitability to electrical stimulation is lost, but further work with different technique would be necessary to establish definitely this contention.

After eserine the magnesium "curarized" denervated muscle gives the usual response to ACh., thus differing again from curare poisoning (Rosen-

blueth and Luco) and lending further support that curare and magnesium exert their effects by entirely different mechanisms.

SUMMARY

Intravenous $MgSO_4$ causes a depression of twitch height in denervated skeletal muscle of dogs. It is concluded that this reduction of contraction height is due to an increase of the threshold of the denervated muscle to electrical stimulation.

Magnesium furthermore temporarily reduces or may abolish the sensitivity of the denervated muscle to ACh., but leaves unaffected the ACh. response subsequent to eserinization of the animal.

Because of the direct action of magnesium upon the muscle there are certain differences between the action of this substance and that of curare.

We gratefully acknowledge the suggestions and constructive criticisms of Dr. T. E. Boyd.

REFERENCES

(1) Meltzer, S. J. and J. Auer. This Journal **14:** 366, 1905.
(2) Lubinska, L. Arch. int. Physiol. **41:** 456, 1935.
(3) Maaske, C. A., T. E. Boyd and J. J. Brosnan. J. Neurophysiol. **1:** 332, 1938.
(4) Boyd, T. E., J. J. Brosnan and C. A. Maaske. J. Neurophysiol. **1:** 497, 1938.
(5) Rosenblueth, A. and J. V. Luco. This Journal **120:** 781, 1937.
(6) Ashkenaz, E. W. J. Cell. and Comp. Physiol. **11:** 163, 1938.
(7) Schmitt, O. H. A. and F. O. Schmitt. Science **76:** 328, 1932.
(8) Brown, G. L. J. Physiol. **89:** 438, 1937.
(9) Blair, H. A. This Journal **124:** 372, 1938.
(10) Knowlton, G. C. and H. M. Hines. This Journal **120:** 757, 1937.

THE RELATIONSHIP BETWEEN VASCULAR VOLUME AND BLOOD FLOW IN THE HIND LIMB OF THE DOG[1]

J. P. HOLT AND HAMPDEN LAWSON

From the Department of Physiology, University of Louisville School of Medicine

Received for publication July 17, 1939

It is generally agreed that in organs whose volume is free to change, vasoconstriction, with reduction in the flow of blood, is accompanied by a reduction in the volume of the organ; and conversely, that vasodilatation, with an increase in the flow, is accompanied by an increase in the volume of the organ. There is no agreement, however, on the essential relationship between the volume change in the organ and the vasomotor change. Since volume changes accompany vasomotor changes in most vascular beds, it may be argued that the two are inseparable. Most workers on the cerebral circulation, in their application of the Monro-Kellie doctrine, have implied that since the volume of blood within the cranium is relatively constant the total flow is governed principally, if not entirely, by the head of pressure.

Although it is no longer questioned that the intracranial vessels are capable of vasomotor activity (Wiggers, 1905; Finesinger and Putnam, 1933; Schmidt, 1934; Gibbs and Lennox, 1935; Forbes and Cobb, 1938; and others), the evidence at hand suggests that these vasomotor changes are of relatively little importance in controlling total flow through the brain (Forbes and Cobb, 1938). No serious attempt has been made, however, to determine whether the relative inadequacy of local vasomotor mechanisms for the total intracranial circulation is due to peculiarities of the vascular bed itself, or to the fact that it is made more or less rigid by enclosure in the cranial cavity, as implied in the Monro-Kellie doctrine.

Since the cranial cavity is not completely rigid (Hill, 1895), and since the irritability of the cranial vessels is also questionable, it was thought that the question might better be studied in other vascular areas. Attempts were made, therefore, to determine whether enclosing the hind limb in a rigid container, so as to keep the volume of the limb constant, reduced the vasomotor response of the limb to constrictor and dilator drugs.

METHODS AND RESULTS. Dogs anesthetized with ether or sodium bar-

[1] A preliminary report of this work was given at the Toronto meeting of the American Physiological Society, 1939.

bital were used. Blood flow was measured in the hind limb by a differential manometer method previously described (Lawson and Holt, 1939), by a modification of the method of Wagoner and Livingston, or by direct measurement of venous outflow.

The limb was usually tightly ligatured with wire at the level of the upper or middle third of the femur, the femoral artery and vein remaining outside

TABLE 1

EXPERIMENT NUMBER	B.F.	R.P.	F.P.	RESTRICTED			UNRESTRICTED		
				Flow change	Per cent flow change	Vol. change	Flow change	Per cent flow change	Vol. change
				cc./min.		*cc.*	*cc./min.*		*cc.*
1	V.O.	A	Sciatic section	+2.5	+29.5				
2	V.O.	A	Sciatic section	+1.9	+86.4		+34.3	+59.2	
3	D.M.	A	Ng.	+24.0	+39.8				
4	D.M.	A	Ng.	+30.0	+125.0				
5	D.M.	A	Ng.	+12.0	+33.3	0			
6	D.M.	B	Epin.	−80.0	−100.0	0	−70.0	−85.8	−8.0
	D.M.	B	Ng.	+60.0	+50.0	0	+60.0	+60.0	+1.0
7	D.M.	B	Epin.	−1.0*		0	−1.6*		−2.5
8	D.M.	B	Epin.	−0.8*		−0.3	−0.5*		−2.4
9	D.M.	B	Epin.	−3.3*			−3.0*		
	D.M.	B	Ng.	+2.1*			+1.6*		
10	W.L.	B	Epin.	−32.0	−76.0	0	−29.0	−76.0	−3.2
11	W.L.	B	Epin.	−10.0*		+0.3	−12.0*		−4.0

Blood flow and limb volume changes in limbs free to change in volume (unrestricted) and in limbs having their volume fixed (restricted). B.F., method of measuring blood flow (V.O., direct venous outflow; D.M., differential manometer method; W.L., modification of the Wagoner and Livingston method). R.P., procedure used to restrict limb volume (A, limb in cast; B, limb bandaged and fixed with negative pressure). F.P., procedure used to change blood flow (Ng., injection of spirits of nitroglycerine; Epin., injection of epinephrine). In experiment 2 the unrestricted flow is the flow in the opposite limb which was not in a cast. Flow changes marked * are deflections of the flow meter, no calibrations for actual flow being available.

the ligature. Procedures for modifying flow through the limb were adopted which had no or irrelevant effects on carotid blood pressure. Reduction in flow was obtained by injection of 0.02 mgm. epinephrine in 1 cc. 0.9 per cent NaCl into the central stump of any convenient branch of the femoral or external iliac artery. Increase in flow was obtained by section of the sciatic nerve in lightly anesthetized animals, or by intra-arterial injection (as with epinephrine) of 2 cc. 1:20 dilution of U.S.P. spirits of nitroglycerine.

In five acceptable experiments (expts. 1 to 5, table 1) an attempt was

made to prevent the limb volume from changing by applying a tight plaster-of-Paris cast to the shaved limb. In experiment 5 a plethysmograph placed over the cast showed no change in the volume of the cast during the blood flow change. Since it may have been possible for the limb to change its volume inside the cast additional data were sought.

In six experiments (expts. 6 to 11, table 1) the limb was wrapped with a porous bandage and placed in a plethysmograph which was sealed to the skin with heavy wire around the upper end of the plethysmograph. The wire shut off all circulation through the limb except that through the femoral artery and vein which were protected from the pressure of the wire by a slotted brass tube. A wire was passed around the base of the foot to stop all blood flow through it. Sub-atmospheric pressures of 245 to 500 mm. Hg were applied in the plethysmograph to fix the limb volume. The negative pressure held the skin tight against the bandange; the limb could not increase in volume due to the bandage and could not decrease due to the negative pressure. In experiments 6 to 9, table 1, the volume of the limb was measured by connecting the plethysmograph to a mercury manometer, the open arm of which was connected to a volume recorder. As shown in the table, flow changes were accompanied by insignificant changes in volume. Since, however, the mercury manometer may have prevented small volume changes of the limb from being transmitted to the volume recorder, in experiments 10 and 11, table 1, the volume recorder was connected directly to the plethysmograph and placed inside a chamber in which the pressure was lowered to that of the plethysmograph, thus leaving the volume recorder free to record changes in limb volume as shown in the table.

Fixation of total limb volume by any of these methods failed to modify significantly the change in blood flow produced by epinephrine, nitroglycerine, or denervation (table 1). Figure 1 presents a typical comparison of the response to epinephrine in the normal limb and in the limb with volume fixed.

Discussion. Except in a few studies (Richards and Plant, 1922) no consideration has been given to the site of the volume changes which normally accompany changes in blood flow. The present data show that in the vascular bed studied a change in total vascular volume is not an essential vasomotor phenomenon. It is assumed that the changes in flow elicited are due to changes in size of some part of the stream-bed. Our methods of fixing total limb-volume do not seem to have prevented or significantly reduced the changes in size of these essential segments. It is obvious that under these conditions, equal and opposite changes in the size of the stream-bed must occur in other segments. Our data suggest, furthermore, that these compensating segments contribute relatively little to the total peripheral resistance. They are probably, therefore, well

down-stream from the arterioles. In the limb whose volume is free to change, they probably have their cross-section reduced during vasoconstriction. In the limb with total volume fixed, they have their cross-section increased during vasoconstriction.

If these data may be transferred to the cranial circulation, it would seem that the fact that the volume of the cranial contents is relatively constant, as stated by the Monro-Kellie doctrine, is of little hemodynamic importance.

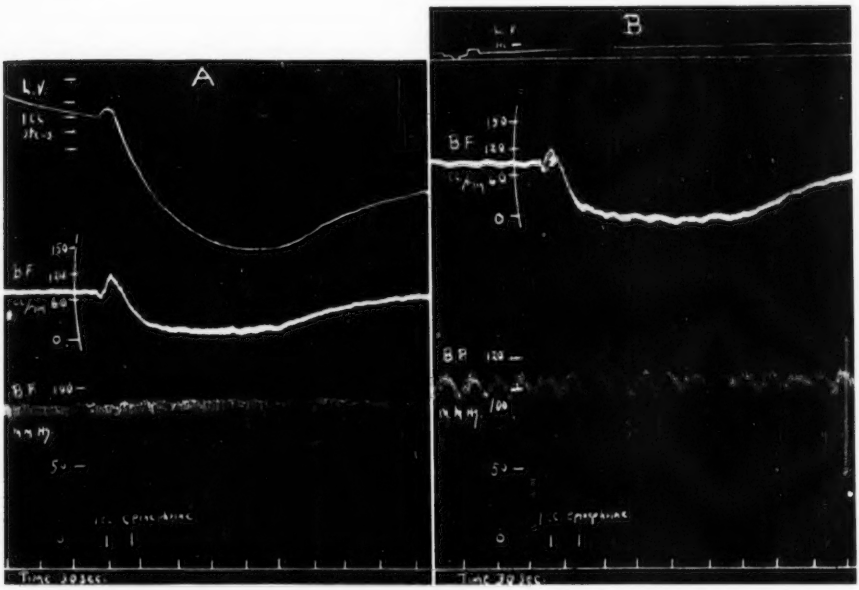


Fig. 1. The effect of intra-arterial epinephrine (between signals) on limb volume and blood flow. *A*, limb free to change its volume. *B*, limb with volume fixed by a negative pressure of 245 mm. Hg. *L.V.*, limb volume (calibration in cubic centimeters). *B.F.*, blood flow in cubic centimeters per minute. *B.P.*, carotid blood pressure. Time in 30 second intervals.

SUMMARY AND CONCLUSIONS

The blood flow through the hind limb of dogs anesthetized with ether or sodium barbital was measured by the direct venous outflow method, a differential manometer method, and a modification of the method of Wagoner and Livingston.

Intra-arterial epinephrine decreased, and intra-arterial nitroglycerine increased the flow. The change in flow with these drugs was approximately the same with the limb free to change its volume and with the

volume fixed. It is concluded that the Monro-Kellie doctrine is of no significance for vasomotor reactions in this vascular area.

REFERENCES

Bayliss, W. M., L. Hill and G. L. Guilland. J. Physiol. **18:** 334, 1895.
Finesinger, J. and T. J. Putnam. Arch. Neurol. and Psychiat. **30:** 775, 1933.
Forbes, H. S. and S. S. Cobb. Brain **61:** 221, 1938.
Gibbs, F. A., E. L. Gibbs and W. G. Lennox. This Journal **111:** 557, 1935.
Lawson, H. and J. P. Holt. J. Lab. and Clin. Med. **24:** 639, 1939.
Schmidt, C. F. This Journal **110:** 137, 1934.
Wagoner, G. W., and A. E. Livingston. J. Pharmacol. and Exper. Therap. **32:** 171, 1928.
Wiggers, C. J. This Journal **14:** 452, 1905.

FURTHER INVESTIGATIONS CONCERNING THE EFFECTS OF FORMALIN ON THE GONADOTROPIC AND THYROID-STIMULATING HORMONES OF CATTLE ANTERIOR PITUITARY GLANDS IN THE GUINEA PIG[1]

LEO LOEB AND S. J. HAYWARD

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine, St. Louis

Received for publication June 21, 1939

In a preceding paper we reported on experiments in which were defined some of the conditions under which formalin diminishes or suppresses the luteinizing and atresin effects normally exerted if a single cattle pituitary is implanted into a guinea pig and, instead, leads to the production of fully mature follicles and produces heat changes (1, 2). There remained some variable factors which needed further analysis, such as the concentration of formalin solutions and the kind of cattle gland used. Furthermore, there was the problem as to the relation between the changes produced in the cattle pituitary by various experimental procedures before implantation and the changes in the ovary caused by implantation of these glands; there remained in addition the problem as to the relation between the ovarian changes and the subsequent changes in the vagina. It was also important to determine under what conditions it was possible to preserve this heat-producing effect of individual cattle pituitaries for longer periods of time, so that glands able to transmit such action would always be on hand for experimental purposes. Lastly, the relation between the effect of formalin on the thyroid-stimulating and gonadotropic hormones needed further analysis.

We carried out 103 additional experiments, in which in each case a single cattle gland was immersed in formalin solutions of different concentrations with differences in pH for various periods of time. The methods used in these experiments are very similar to those applied in the preceding paper (2).

The main results are represented in table 1, in which are shown the concentration of the formalin used, the pH of the solution, the number of implantations, and the number of positive, intermediate and negative results obtained.

[1] These investigations were carried out with the aid of grants from the International Cancer Research Foundation and from the Committee on Research in Endocrinology of the National Research Council.

Positive results are those in which the ovaries contain fully mature follicles, which induce heat, as indicated by the marked enlargement of the vagina and the hyperemia of the uterus. The intensity of the heat

TABLE 1

PER CENT OF FORMALIN	pH	NUMBER OF IMPLANTATIONS	NUMBER OF CASES		
			Positive	Intermediate	Negative
Series I					
0.25 (2 cases 0.15)	5	4	19	1	7
Series II					
0.25	8	4	6	2	5
Series III					
0.5	5	4	0	1	1
0.5	8	4	0	0	7
Series IV					
0.25	10	4	0	3	3
0.25 (1 case 0.5)	12	4	0	0	6
Series V					
Immersion in formalin followed by glycerin					
0.25	5 or 5-6	2	3	0	1
0.5	5 or 5-6	2	4	3	0
Series VI					
0.75	5	4	5	0	4
0.75	8	4	0	0	4
Series VII					
1	5	4	0	0	2
1.5	8	4	0	0	2
2	5	4	0	0	2
2	8	4	0	0	2
2.5	8	4	0	0	6
3	5	4	0	0	3
4	5	4	0	0	1

Total, 103 cases; 37 positive, 10 intermediate, 56 negative.

changes can only be graded by a study of microscopic sections of the vagina. In addition to the maturation of follicles there may be luteal changes in the ovaries of some animals, while in others they are lacking;

but they are always reduced in intensity after formalin treatment of the pituitaries. The principal luteal changes consist in early stages of pseudocorpora lutea (pseudolutein bodies), which develop without a preceding ovulation, as a result of the ingrowth of capillaries and connective tissue into a mature or maturing follicle. In addition we may find luteinization of theca interna, which is most marked in follicles which are in the later stages of atresia, and lastly, enlargement of interstitial gland cells, which are chiefly derived from remnants of theca interna of atretic follicles in the medulla of the ovary. For a further analysis of these changes in the ovary we may refer to a previous paper (3).

Negative results are those in which mature follicles or pseudolutein bodies are lacking in the ovaries; also luteinization of theca and interstitial gland are usually lacking, or at least much reduced. Vagina and uterus are not enlarged and as a rule the vagina is very thin and, as microscopic sections show, in a resting state; but in some cases there may be a very slight stimulation of the vagina.

Intermediate cases are those in which partially mature or even completely mature follicles are found, but in which their action is more or less neutralized by the strong development of luteal structures. In other cases there is a partial maturation of follicles with or without the presence of luteal structures. Certain transitions exist between these types of ovaries. The vagina is usually in an intermediate condition.

The various conditions found in the ovaries and vagina are represented by Roman figures as follows:

Changes in ovary. I = mature follicles, no pseudolutein bodies, no or only very slight luteinization of theca or interstitial gland. II = mature follicles, no pseudolutein bodies, some luteinization of theca. III = mature follicles, pseudolutein bodies, slight luteinization of theca. IIIa = mature follicles, pseudolutein bodies, much or moderate luteinization. I-, II-, IIIa- = the ovarian changes indicated by the numbers but associated with incomplete maturation of follicles. IV = lack of mature follicles, but pseudolutein bodies and some theca luteinization are found. V = no mature follicles, no pseudolutein bodies, some theca luteinization. Va = no mature follicles, no pseudolutein bodies, no or very little theca luteinization.

Changes in vagina. I = squamous epithelium covered with a layer of keratin. II = squamous epithelium without a covering of keratin. III = partial formation of squamous epithelium covered by a layer of vacuolated high cylindrical cells arranged in papillae. IV = very slight proliferation of cuboidal epithelium. V = resting vagina.

Seven series of experiments were carried out. Series I, 0.25 per cent (in 2 experiments 0.15 per cent) formalin solution, pH 5; series II, 0.25 per cent formalin solution, pH 8; series III, 0.5 per cent formalin solution, pH 5 or pH 8; series IV, 0.25 per cent formalin solution, pH 10 and pH 12;

series V, 0.25 per cent or 0.5 per cent formalin solution followed by immersion in glycerin for various periods of time, pH 5 to 6; series VI, 0.75 per cent formalin solution, pH 5 or pH 8; series VII, 1 per cent, 1.5 per cent, 2 per cent, 2.5 per cent, 3 per cent, 4 per cent formalin solutions, pH 5 or pH 8.

As a rule the time of immersion in the solution was four or seven days at 37 to 39°C., but in some experiments this period was followed by variable periods, during which the pieces of hypophyseal gland were immersed at room temperature either in the same formalin solution or in glycerin. The number of implantations into each guinea pig as a rule was four, one implantation of a fourth of a gland being made on four successive days, the examination taking place on the fifth day; but in series V one-fourth of a gland was implanted on each of two successive days and examination took place on the fourth day. Each gland was cut into four or sixteen pieces previous to immersion; pieces the size of one-sixteenth or one-thirty-second part of a gland were used for implantation.

Results. These experiments confirm the conclusions stated previously that approximately pH 5 (pH 4–pH 6) of the formalin solutions for immersion of the cattle glands is the most favorable one for the production of mature follicles and for reduction or suppression of the luteinizing effects in the ovaries of guinea pigs, and for the initiation of heat changes. With solutions of formalin kept at pH 5, 31 (63 per cent) positive results, 5 (9 per cent) intermediate results, and 13 (28 per cent) negative results were obtained. With pH 8 in the corresponding series there were 6 (25 per cent) positive results, 2 (8.3 per cent) intermediate results, and 16 (66.7 per cent) negative results. If we combine these figures with those obtained in our earlier investigations, with which in a general way they are in agreement, the results are as follows: pH 5: 47 positive, 11 intermediate, 21 negative cases; pH 8: 10 positive, 7 intermediate, 20 negative cases. These figures make it very probable that the optimum pH is about 5. At pH 10 no positive, but a few intermediate results were obtained in addition to the negative ones, which represented the majority. These findings likewise are in agreement with our earlier ones. At pH 12 only negative results were obtained. Experiments with pH 2 were not repeated; there is reason for assuming that at this pH negative results predominate, in accordance with our previous findings.

In these investigations we studied on a somewhat larger scale the effects of different concentrations of the formalin solutions which were used for immersion of the cattle glands. Positive and intermediate results were obtained in 0.25 per cent, 0.5 per cent and 0.75 per cent formalin solutions at pH 5. At pH 8 the results in 0.5 per cent and 0.75 per cent formalin were negative.

In series V the cattle pituitaries after having been immersed in 0.25

per cent or 0.5 per cent formalin solutions for four or seven days were then transferred to glycerin for various periods of time. Only two implantations on two successive days took place as a rule and examination occurred on the fourth day. With both 0.25 per cent and 0.5 per cent positive results were obtained under these conditions. With a 0.75 per cent concentration of formalin (series VI) positive results were also obtained but they were less numerous than with a concentration of 0.25 per cent. On the other hand, in series VII with 1 per cent (2 experiments), 1.5 per cent, 2 per cent, 2.5 per cent, 3 per cent and 4 per cent concentrations of formalin, all results were negative; these concentrations are too strong for preservation of the maturation hormone in the cattle glands. In our former investigations, although some positive results were observed with a 1 per cent concentration, negative results preponderated.

The optimal concentration of formalin in these experiments was 0.25 per cent and the optimal pH was 5; the optimal number of days of immersion was seven. In experiments carried out under these conditions during the winter months positive results were found in the large majority of cases.

There is reason for assuming that the glands of different kinds of cattle (heifer, steer, cow, bull) differ as far as the relative distribution of the follicle growth and maturation, luteinizing, and thyroid-stimulating hormones is concerned. While in the large majority of our experiments we used heifer pituitaries, in a number of cases we tested also steer and cow glands and obtained some positive results. However, while positive results may be obtained with steer and cow glands as well as with heifer pituitaries, the number was not as large with steer as with the use of heifer pituitaries.

The initial weights of the guinea pigs in most cases varied between 180 and 192 grams. There was usually a slight gain in weight during the experiments; in some animals the weight remained about the same, and in a small minority there was a slight loss. There was no noticeable difference in the changes in weight in experiments with positive, intermediate and negative results.

As stated, in the large majority of experiments the pituitary glands were implanted into guinea pigs after they had been immersed in the formalin solution for periods of four or seven days. But in certain instances the glands after having been exposed to the action of the formalin solution at a temperature of 37–39°C. for the stated time, were subsequently kept at room temperature, either in the same formalin solution or in glycerin, 5 cc. of glycerin being added to each gland. In series I two positive results were obtained after the cattle pituitary had been immersed first in a 0.25 per cent or 0.15 per cent formalin solution for seven days at 37°C. and then kept in the same formalin solution at room tem-

perature for three weeks longer. In other experiments in which the pieces were first immersed in a 0.25 per cent or 0.15 per cent formalin solution for four or seven days at 37°C. and then kept for periods of 7, 14 or 21 days in the same formalin solution at room temperature, the results were negative. In series VI two experiments in which the pieces were kept first in a 0.75 per cent formalin solution for four days at 37°C. and then for two weeks at room temperature, the results were negative. Likewise in series VII in three experiments in which the pieces were kept in 1 per cent or 2 per cent formalin solution for seven days at 37°C. and then for seven days or three weeks at room temperature, the results were negative.

In series V the glands were kept first for periods of four or seven days in 0.25 per cent or 0.5 per cent formalin solution and subsequently in glycerin at room temperature for periods of 1, 8, 30, 65 or 112 days, 5 cc. of glycerin being added to each gland. In seven such experiments positive results were obtained, in three additional experiments the results were intermediate, and in one experiment, in which the gland had first been kept for four days in a 0.25 per cent formalin solution at 37°C. and subsequently for 30 days at room temperature in glycerin, a negative result was obtained.

We may then conclude that it is possible to preserve the glands, modified by treatment with formalin in such a way that they cause the production of mature follicles, with diminution or suppression of the luteinizing effects and induce estrus in the guinea pig, for at least four months and probably for a much longer period by immersing them in glycerin following the treatment with formalin.

If we analyse the causes of the positive, intermediate and negative results in the various experiments, we find that in all cases but one the negative results were due to too strong an action of the formalin, which led to a complete or almost complete destruction of all the gonadotropic hormones. The one exceptional case occurred in series V,—four days' immersion in 0.25 per cent formalin at pH 5, followed by immersion in glycerine for 30 days; in this experiment there was associated with a lack of mature follicles the formation of pseudolutein bodies and very marked luteinizing processes in theca; the vagina was in a resting condition.

There is a correlation between the changes produced by the treated pituitaries in the ovaries and in the vaginae of the guinea pigs, as shown by the microscopic study of the latter organs. Out of 55 negative cases the ovaries conformed to type Va in all but two; mature follicles, pseudolutein bodies, as well as luteinization of theca and interstitial gland were lacking. In one case in series I there was a slight luteinization of theca, and in one case in series IV (0.25 per cent formalin solution at pH 12) there was beginning maturation in a follicle. In 34 of these cases sections of the vagina were studied. In 27 cases the vagina was resting (grade V);

in 5 cases there was a very slight proliferation in the cuboidal epithelium, as evidenced by the finding of some mitoses, or by a slight increase in the cuboidal cell layers (grade IV). In 2 cases the proliferation went somewhat farther (grade III or III–IV). Out of the five animals with grade IV in vagina, in only one slight luteinization of theca was found. The slight stimulating action in these few instances is probably due to the activity of good-sized, although immature follicles. In the two cases in which the vagina showed a somewhat stronger stimulation (grade III or III–IV) very large, although immature preserved follicles were observed. In one of these two cases luteinizing processes were lacking altogether, in the other they were very slight. These two experiments represent a transition from the negative to the intermediate group.

Out of the 46 experiments with positive or intermediate results the vagina was microscopically studied in 38 cases. The correlation between the conditions found in ovaries and vagina was as follows: *Group A.* Ovary I or I–II: vagina I, 13 cases; vagina II, 2 cases; vagina III or II–III, 3 cases. *Group B.* Ovary II: vagina I or I–II, 4 cases; vagina II, 2 cases; vagina III or II–III, 3 cases. *Group C.* Ovary IIIa: vagina I, 0 cases; vagina II, 1 case; vagina III or II–III, 4 cases. *Group D.* Ovary I- or (I–II)-: vagina I or II, 0 cases; vagina III or II–III, 6 cases. Combining *Groups B and C.* Ovary II or IIIa: vagina I or I–II, 4 cases; vagina II, 3 cases; vagina III or II–III, 7 cases.

The effect of formalin on the thyroid-stimulating hormone. In all but 9 of the 103 animals examined the thyroid-stimulating hormone had been destroyed. In nine guinea pigs some hypertrophy of the thyroid gland was effected. Among them there were four with positive results in the sex organs; in two of these the thyroid hypertrophy was just beginning or slight; in one it was moderate and in one it was marked. In the two last named experiments the thyroid hypertrophy was associated with formation of mature follicles and suppression of luteinizing effects in the ovaries. In the intermediate cases and in two of the negative cases the hypertrophy was very slight; in the two remaining animals it was moderate; in one of these the pituitary gland had been exposed to a 2 per cent solution of formalin at pH 8 for seven days.

In former investigations we found that very often, but not in every case, the thyroid-stimulating hormone was associated with luteinizing and atresin effects in the ovaries, but there were cases in which the thyroid-stimulating hormone was active, while the luteinizing hormone had been destroyed and the follicular growth and maturation hormone preserved. Our present investigations confirm the conclusion that the thyroid-stimulating hormone and the substances causing luteinization and atresin effects in the ovaries are not identical.

SUMMARY

1. Immersion of single cattle pituitary glands in formalin solutions causes a marked reduction or complete suppression of the luteinizing and atresin effects and in many instances a full maturation of follicles in the ovary of the guinea pig; the mature follicles induce heat changes. The largest number of positive results were obtained if heifer pituitaries were immersed in 0.25 per cent solutions of formalin at pH 5 for seven days.

2. Negative results in all except one experiment were due to too strong an action of formalin, which led to the destruction of all the gonadotropic hormones.

3. The maximum estrogenic effects in the vagina, as a rule, were obtained in those experiments in which mature follicles were produced and in which at the same time the reduction of the luteinizing effects, consisting in formation of pseudolutein bodies, theca luteinization and production of interstitial gland, was most complete. There is some indication that these luteinizing effects counteracted the heat changes which were produced by the mature follicles. It is probable, however, that a slight stimulation of the vagina may be produced by large but not yet mature follicles.

4. A definite reduction in the luteinizing effects in the ovaries of the guinea pigs is a prerequisite for the production of fully mature follicles and of the typical heat changes in the secondary sex organs.

5. It is possible to maintain cattle pituitary glands, which have been converted by the action of formalin into maturation-producing and estrogenic agents in the sex organs of the guinea pig, unchanged for at least four months, but presumably for even longer periods by immersion in glycerin.

6. The thyroid hormone is injured or destroyed by formalin. This action is independent of the luteinizing or atresin effects which are produced in the ovaries. This confirms the conclusion that luteinizing, atresin and thyroid-stimulating actions are not due to the same agent.

REFERENCES

(1) Loeb, L., J. Saxton and S. J. Hayward. Endocrinology **20:** 511, 1936.

(2) Hayward, S. J., J. H. Pollock and L. Loeb. This Journal **125:** 113, 1939.

(3) Loeb, L. The structural changes which anterior pituitary hormones produce in the ovary of the guinea pig. Volume dedicated to R. T. Frank. C. V. Mosby Company, June 1937.

THE INFLUENCE OF AIR MOVEMENT UPON HEAT LOSSES FROM THE CLOTHED HUMAN BODY

C.-E. A. WINSLOW, A. P. GAGGE AND L. P. HERRINGTON

Contribution no. 25, from the John B. Pierce Laboratory of Hygiene, New Haven, Conn.

Received for publication June 28, 1939

OBJECTIVES AND GENERAL TECHNIQUE. In earlier communications from this laboratory we have discussed thermal interchanges between the nude and clothed human body and its environment, as analyzed by the procedures of partitional calorimetry. In two of these studies (Winslow, Herrington and Gagge, 1936; Gagge, Herrington and Winslow, 1937) we have specifically considered the factor of air movement. We then pointed out that there was no agreement as to the type of physical formula which expressed the influence of air movement upon the heat loss from the human body. One formula in common use assumed the relationship to be linear, another considered heat loss as proportional to the square root of air velocity. We found that with relatively low air velocities (from 0 to 50 cm. per sec.) either of these formulae expressed the results with approximately equal accuracy if the entire range was considered; and we, therefore, used the square-root formula for purposes of convenience. It is clear, however, that for velocities below 20 cm. per second, which are of special practical interest, the formulae do not agree as well as they do for average results over a wider range. The primary purpose of the present study was to settle the point in question by using higher air velocities and continuing the experiments for longer periods of time. In their course some light was, incidentally, thrown upon the gradual physiological adaptation of the body to a cool environment.

The experiments here reported were made with two subjects whose physical characteristics have been described in an earlier communication (Gagge, Winslow and Herrington, 1938). The studies were made in our experimental copper booth with the subjects lightly clothed and in a semi-reclining position. Other experimental details will be found in the papers cited above. Relative humidity was kept approximately constant (between 40 and 50 per cent of saturation) and no attempt was made to produce an air-wall differential.

Three sets of experiments were conducted, with the following conditions of air temperature and air movement:

Series A, temperature 16.1°C.; air movement, 4.6 cm. per second
Series B, temperature 19.2°C.; air movement, 34.0 cm. per second
Series C, temperature 22.8°C.; air movement, 264.0 cm. per second

These combinations of air temperature and air movement were selected to produce approximately similar rates of heat loss from the body, as predicted from our earlier studies. As will be noted later, the conditions of series A actually removed least heat from the body, and those of series B removed most, as compared with the intermediate cooling effect of series C.

These differences in heat removal are small and indicate that the pre-experimental computations were successful in determining the increases in air temperature which would approximately balance the additional cooling effect of the two higher air movements.

Process of adaptation to a cool environment. The direct observations recorded are summarized in table 1. Each column of this table represents the mean for the time indicated of three or four experiments with each of the two subjects, or six to eight experiments in all. Values for rectal temperature and mean skin temperature have been smoothed by averaging successive observations in groups of three. Surface temperature readings were taken by the thermopile at 15 points, 3 on the exposed surfaces of the head and 1 on the exposed surface of the hand, 2 on the clothing of the arm, 4 on the clothing of the trunk, and 5 on the clothing of the leg. Thermocouple records of skin temperature under the clothing were made at the last 11 points. In determining mean clothing temperature and mean skin temperature for the whole body, the head readings received a weight of 7, the upper extremity readings a weight of 21, the trunk readings a weight of 31, and the lower extremity readings a weight of 41. The readings on the head and hand appear in both mean clothing temperature and mean skin temperature. The net results of our direct primary observations are presented in figure 1.

All three curves for metabolism show a fall of 2.5 to 4.5 kilogram-calories during the first two hours of the experiment with a subsequent rise which, in two of the three cases, reaches or exceeds the original value. This is the phenomenon recently described by Berkson and Boothby (1938). We are inclined, with them, to explain the early fall as due to relaxation of the subjects in the semi-reclining position and the later increase to a slight degree of restlessness.

The temperature of the skin of the trunk under the clothing (T_T), shows, in series A and C, only a very slight decrease, from 35.5° or 36.0° to 35°, and reaches its stable level at 35° in about 80 minutes. In series B, the initial trunk temperature was close to 35° and almost no change occurred.

TABLE 1

Progressive reactions of the clothed human body to a cool environment

ELAPSED TIME	T_A*	T_{Cl}	T_H	T_U	T_T	T_L	T_S	T_R	M
				Series A—4.6 cm./sec.					
minutes	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	*kg.-Cal./ sq. m.-hr.*
0			30.4	33.1	35.9	33.1			
20			30.2	32.4	35.3	32.8	33.45	37.07	53.7
40	16.2	26.0	30.1	31.8	35.4	32.6	33.18	37.04	53.0
60		25.9	29.8	31.7	35.3	32.3	32.93	37.01	51.7
80	16.1	25.8	29.9	31.2	35.2	32.1	32.75	36.97	50.5
100		25.7	29.9	30.8	35.2	31.9	32.54	36.93	50.3
120	16.1	25.6	29.9	30.5	35.4	31.6	32.36	36.89	50.2
140		25.3	29.6	30.2	35.4	31.3	32.21	36.86	50.8
160	16.2	25.1	29.3	29.9	35.1	31.1	31.87	36.82	51.4
180		25.1	29.2	29.9	35.1	30.8	31.72	36.81	52.3
200	16.1	25.0	29.3	29.6	35.1	30.6	31.60	36.76	53.3
				Series B—34 cm./sec.					
0			28.9	31.6	35.2	32.6			
20	19.7	25.2	28.4	31.0	35.1	32.7	32.74	36.97	53.4
40	19.4	24.9	28.1	30.4	34.9	32.6	32.58	36.95	53.0
60	19.2	24.8	27.8	30.2	34.9	32.4	32.36	36.93	51.8
80	19.2	24.5	27.6	29.9	34.7	32.1	32.18	36.88	51.7
100	19.1	24.3	27.4	29.6	34.7	31.9	31.98	36.83	51.6
120	19.1	24.2	27.2	29.5	34.7	31.6	31.83	36.79	51.1
140	19.1	24.1	27.3	29.3	34.7	31.3	31.68	36.75	51.0
160	19.1	24.2	27.3	29.1	34.8	31.0	31.56	36.72	52.0
180	19.2	24.2	27.3	29.0	34.8	30.9	31.47	36.68	53.6
200	19.1	24.2	27.3	28.8	34.9	31.0	31.43	36.64	55.0
220	19.2	24.1	27.3	28.7	35.1	30.7			
				Series C—264 cm./sec.					
0			28.8	32.4	35.7	33.4			
20	22.8		28.6	31.4	35.4	33.1	33.18	37.09	55.1
40	22.7	25.4	28.3	30.8	35.2	32.7	32.83	37.07	54.7
60	22.6	25.4	28.2	30.6	35.1	32.4	32.59	37.03	54.0
80	22.6	25.3	28.1	30.4	35.1	32.1	32.38	36.99	53.0
100	22.6	25.3	28.0	30.2	34.9	31.8	32.20	36.96	52.1
120	22.7	25.3	27.9	30.1	34.8	31.7	32.04	36.91	51.3
140	22.7	25.2	27.9	30.0	34.8	31.4	31.94	36.84	50.7
160	22.7	25.1	28.0	29.9	34.8	31.2	31.83	36.80	50.9
180	22.8	25.1	27.9	29.9	34.8	31.0	31.74	36.74	51.2
200	22.8	25.1	27.9	29.8	34.8	30.9	31.68	36.70	51.5
220			27.9	29.8	34.8	30.9			51.8

*T_A, ambient air temperature; T_{Cl}, weighted mean temperature at fifteen points on head, hands, and clothing surfaces; T_H, mean skin temperature of head; T_U, mean skin temperature of upper extremities; T_T, mean skin temperature of trunk, T_L, mean skin temperature of lower extremities; T_S, weighted mean of T_H (7), T_U (21) T_T (31), and T_L (41); T_R, rectal temperature; M, metabolism.

The surface of the head (T_H) and the total surface of the skin (head and hands) and clothing exposed directly to the air (T_{cl}) drop more rapidly and reach a level (except in series A, where the initial value was high) after 120 to 160 minutes. It should be noted that determinations of T_{cl} began only after 40 minutes, and, no doubt, a considerable fall occurred prior to this time.

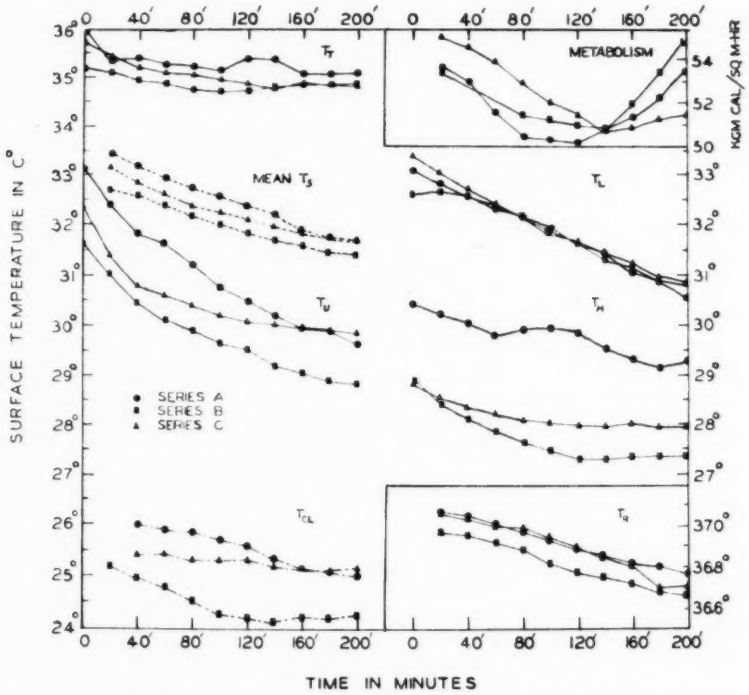


Fig. 1. Progressive temperature and metabolic response of the clothed body over a period of 200 minutes for three environmental conditions.

Series A: dry bulb, 16.1°C; air movement, 4.6 cm. per second. Series B: dry bulb, 19.2°C; air movement, 34.0 cm. per second. Series C: dry bulb, 22.8°C; air movement, 264.0 cm. per second.

Rectal temperature (T_R), mean skin temperature of the exposed hands and head and the other areas of skin under the clothing (T_S), temperature of the upper extremities, including the skin of the hands and the skin of the arms under the clothing (T_U), and temperature of the skin under the clothing of the lower extremities (T_L) were still falling between 160 and 200 minutes, although in the case of T_U the curves flatten toward the end.

This was a disappointment to us, since we had hoped, in these experiments, to reach a stable state where storage no longer occurred.

Comparison of the three experimental conditions shows that the condition with an air temperature of 19.2° and an air movement of 34 cm. per second was characterized by lower final values for T_{cl}, T_H and T_v; while the condition with air temperature of 16.1° and an air movement of 4.6 cm. per second was characterized by higher final values for clothing, head, and air temperatures. These are the surfaces wholly or in part directly exposed to the atmospheric environment and the differences noted are related to differences in the actual calorie demand of the environment, as will be shown in a later section.

In comparing the results of the present experiments with minimal air movement with earlier experiments under comparable conditions (Winslow, Herrington and Gagge, 1938) we find that our present studies show a higher mean skin temperature, under the clothing, and a lower head and clothing surface temperature than those recorded in the earlier work. That is, thermocouple readings were higher and thermopile readings lower. So far as skin temperatures under the clothing are concerned, we suspect that differences in the thermocouple set-up may explain the phenomena observed. In our recent studies a stouter and more rigid harness was used to hold the thermocouples and the couples were, therefore, applied more closely to the surface of the skin. Any thermocouple reading must be an approximation. If the couple is held closely against the skin, local heat loss is interfered with, and too high a reading is obtained; if the thermocouple is not applied closely, its reading will be influenced by the air and will be too low (if the true temperature be taken as that which the particular skin area would assume under normal conditions).

On the other hand, we believe that, for corresponding conditions, the readings of clothing surface temperature in our earlier work were slightly high. In these experiments, we noted that the subjects, in taking readings, sometimes held the pile so close to the clothing surface as to interfere with normal heat loss. In our recent work, thermopile readings on the clothing surfaces were made by the observer, not by the subject himself, and always with the pile held 1 cm. away from the skin or clothing surface.

Computation of storage and of heat loss due to convection plus radiation. In our earlier work we have shown that within a certain range of atmospheric conditions, where storage is slight, changes in rectal temperature truly represent changes in body temperature as a whole (Gagge, 1936). In this region, storage can be determined from rectal temperature; and, knowing evaporation and storage, radiation constants and convection constants for minimal air movement can be computed. Beyond this region we have, in the past, computed radiation and convection from these constants and estimated storage by difference. Without knowledge of the

exact quantitative influence of air movement upon heat loss we cannot use this procedure for high air movements, since both convection loss and storage are unknown. The present studies, in which observations were continued for 200 minutes, were planned in the hope that a stable state (with zero storage) would be reached, so that the difference between metabolism and evaporation ($M - E$) would equal the sum of radiation and convection ($R + C$). By evaluating R, we could then determine C, and from C find the relation between convection loss and air movement.

Actually, however, as indicated in figure 1, neither skin temperature nor rectal temperature reached a level of stable adjustment in the 200-minute period and the discomfort of the subjects was too great to prolong the experiments further. We were, therefore, forced to compute storage (S) by an assumed weighting of the observed rate of fall of skin and rectal temperature.

The pertinent data for our three series of experiments are presented in table 2, the values being derived from the data in table 1.

In the first and second columns are given observed values for metabolism and evaporation, the latter based on a mean value for the whole period. In the third and fourth columns are shown the falls in mean skin temperature and mean rectal temperature for overlapping 40-minute periods. In the fifth column we have estimated the change in mean temperature of the body tissues as a whole by assigning a weight of 1 to skin temperature change, and a weight of 2 to rectal temperature change. The ratio between skin and rectal temperature, actually representing the mean temperature of the body tissues, may, of course, be different for different environmental conditions. Hardy and DuBois (1938) computed a ratio of 4 for rectal temperature to 1 for skin temperature in the temperature range 23° to 35°C. for nude subjects, while Burton (1935) gives a ratio of about 2 for rectal temperature to 1 for skin temperature for a cooler environment of 23.3°C. with subjects lightly clothed. It is not clear that Burton's condition was cooler on the average for his clothed subjects than Hardy's was for nudes. As a result, however, of the longer periods of observation in Burton's experiments, and the fact that his skin and rectal temperature observations showed changes of the order of those observed by us, his relative weights should fit our situation.

In our own earlier work (Gagge, Winslow and Herrington, 1938), we have shown that for clothed subjects, under cool conditions, the gradient between skin and body temperatures extends inward from the surface for a distance of 2 to 3 cm. From a consideration of our computations and those of similar nature by other investigators (Burton, 1935; Bazett and McGlone, 1927) it appears likely that for our conditions this figure is approximately correct. An outer layer of this depth would represent about half the total mass of the body. Below this layer, the body tissues are essentially at

rectal temperature while the mean temperature of the peripheral area must be intermediate between skin and rectal temperature. Hence, the Burton formula seems reasonable and has been adopted by us. The "weighted

TABLE 2

*Elements of thermal interchange in a cool environment**

PERIOD	M	E	ΔT_S	ΔT_R	WEIGHTED ΔT_B	S	$\frac{M-E}{+S}$ OR $R+C$	R	C	$T_{Cl}-T_A$	k_C	T_S-T_{Cl}	K_{Cl}
						Series A—4.6 cm./sec.							
minutes	*kg.-Cal./sq.m.-hr.*	*kg.-Cal./sq.m.-hr.*	°C.	°C.	°C.	*kg.-Cal./sq.m.-hr.*	*kg.-Cal./sq.m.-hr.*	*kg.-Cal./sq.m.-hr.*	*kg.-Cal./sq.m.-hr.*	°C.	*kg.-Cal./sq.m.-hr.-°C.*	°C.	*kg.-Cal./sq.m.-hr.-°C.*
20-60													
40-80	51.7	10.3	0.43	0.07	0.19	11.4	52.8	32.9	19.9	9.82	2.0	7.05	7.5
60-100	50.5	10.3	0.39	0.08	0.18	10.7	50.9	32.3	18.6	9.66	1.9	6.94	7.3
80-120	50.3	10.3	0.39	0.08	0.18	11.1	51.1	32.2	18.9	9.60	2.0	6.88	7.4
100-140	50.2	10.3	0.33	0.07	0.16	9.7	49.6	31.4	18.2	9.38	1.9	6.83	7.3
120-160	50.8	10.3	0.49	0.07	0.21	12.4	52.9	30.9	22.0	9.21	2.4	6.88	7.7
140-180	51.4	10.3	0.49	0.05	0.20	12.1	53.2	30.3	22.9	9.05	2.5	6.77	7.9
160-200	52.3	10.3	0.27	0.06	0.13	7.7	49.7	29.9	19.8	8.94	2.2	6.66	7.5
											m2.13		m7.45
						Series B—34 cm./sec.							
20-60	52.6	11.3	0.38	0.04	0.15	9.0	50.3	18.6	31.7	5.55	5.7	7.60	6.6
40-80	52.3	11.3	0.40	0.07	0.18	10.7	51.7	18.6	33.1	5.55	6.0	7.60	6.8
60-100	51.7	11.3	0.38	0.10	0.20	12.1	52.5	17.9	34.6	5.33	6.5	7.66	6.8
80-120	51.4	11.3	0.35	0.09	0.18	11.1	51.2	17.3	33.9	5.16	6.6	7.71	6.6
100-140	51.3	11.3	0.30	0.08	0.15	9.0	49.0	16.9	32.1	5.05	6.4	7.66	6.4
120-160	51.5	11.3	0.27	0.07	0.13	8.0	48.2	16.7	31.5	5.00	6.3	7.55	6.4
140-180	52.3	11.3	0.21	0.07	0.12	7.4	48.4	16.9	31.5	5.05	6.2	7.38	6.6
160-200	53.5	11.3	0.13	0.08	0.09	5.7	47.9	16.7	31.2	5.00	6.3	7.33	6.5
											m6.25		m6.60
						Series C—264 cm./sec.							
20-60	54.7	9.9	0.59	0.06	0.23	13.7	58.5	9.1	49.4	2.72	18.1	7.44	7.9
40-80	54.0	9.9	0.45	0.08	0.19	11.7	55.8	9.3	46.5	2.78	16.7	7.22	7.7
60-100	53.0	9.9	0.39	0.07	0.17	10.4	53.5	9.1	44.4	2.72	16.3	7.10	7.5
80-120	52.1	9.9	0.34	0.08	0.17	10.4	52.6	8.9	43.7	2.66	16.4	6.94	7.6
100-140	51.3	9.9	0.26	0.12	0.17	10.1	51.5	8.7	42.8	2.61	16.4	6.77	7.6
120-160	50.7	9.9	0.21	0.11	0.14	8.7	49.5	8.4	41.1	2.50	16.4	6.77	7.3
140-180	50.9	9.9	0.20	0.10	0.13	8.0	49.0	8.0	41.0	2.39	17.2	6.72	7.3
160-200	51.2	9.9	0.15	0.10	0.11	6.7	48.0	7.6	40.4	2.28	17.7	6.66	7.2
											m16.91		m7.51
Col.	1	2	3	4	5	6	7	8	9	10	11	12	13

* For explanation see text.

mean $\Delta T_B''$ in column 5 has, therefore, been computed as

$$\frac{\Delta T_S + 2\Delta T_R}{3}.$$

Column 6, our storage value, is derived by multiplying column 5 by a factor (60.3) representing the product of the mean body weight of the two subjects (divided by their mean surface area) by the specific heat of human flesh, and corrected to an hourly basis from the 40-minute periods tabulated. Column 7 is the algebraic summation of columns 1, 2 and 6.

Radiation coefficients of the copper booth. In our first computations we assumed air temperature and wall temperature as the same, since the contact temperature of the copper walls was, of course, identical with that of the air. Minor anomalies in the results led us to question this assumption and we finally realized that in this peculiar experimental situation (a human subject surrounded by walls reflecting radiant heat) reflection of radiant body heat back to the body itself might be a quantitatively significant factor. A special test was set up in which radiation effects from the copper walls were determined at various angles by a thermopile, with and without the presence of the subject in the booth. This study showed that the radiation temperature of the walls of the booth actually did increase with the presence of the subject in proportion to the difference between clothing temperature and air temperature. The rise was equal to an increase in radiant wall temperature (T_W) of 0.67° for a 10° value of $T_{Cl} - T_A$ (mean surface clothing temperature minus air temperature) within the range studied. The reflection coefficient of the booth in relation to the subject was defined and evaluated as:

$$r = \frac{T_W - T_A}{T_{Cl} - T_A} = 0.067,$$

and the absorption coefficient of the booth for radiation from the subject was 0.933. In any final computation one therefore should use a T_W corresponding to the formula above (varying for the different series from 0.17° for series C to 0.67° for series A above air and contact wall temperature).

The radiation constant. Before computing the radiation heat loss, which must be subtracted from $R + C$ to give convection loss, we thought it desirable to use our new data for a check on the radiation constant—and, in particular, on the radiation area of the human body. In our earlier work this constant was determined by holding air temperature and air movement constant and varying wall temperature. The technique involved in series A, B and C of the present study made it possible to approach the same end in another way. In the experiments here reported we were dealing with a situation in which the temperatures of air and walls were nearly identical, but were varied together while air movement also varied. Air movement influences the convection constant but not the radiation constant. Therefore, if we compute K_O—the rate of heat loss

by radiation plus convection for each degree difference between clothing surface temperature and air temperature ($R + C$ in column 7 of the tables divided by $T_{cl} - T_A$ in column 10)—and then plot these K_o values against some power of air velocity which will yield a linear relationship, the intercept of the graph on the K_o ordinate should give a value for the radiation constant. This has been done in the solid line of figure 2, using the square root of the air velocity which proved to be the appropriate value for linearity. The intercept crosses the K_o ordinate at a value of 3.35. This value, however, is based on $T_{cl} - T_A$. As a result of reflection from the copper walls, described above, this value of the radiation constant must be divided by 0.933, the absorption coefficient of the booth as derived in the previous paragraph, if we desire to compute the true radiation area.

The true value of K_R is, therefore, 3.59, which, when compared with the value derived directly from the Stefan Radiation Law, corresponds to a radiation surface area of 71.5 per cent of the total surface area of the body as found by the DuBois relationship. Our earlier work indicated a radiation area equal to 75.5 per cent of the DuBois area. This lower radiation area may well have been a real difference, associated with the tendency of the subjects to protect themselves against uncomfortably cold conditions by holding the legs together and the arms close to the body.

The convection formula. From figure 2 the slope of the least square fit is 1.043. Therefore, the total convection constant for any air movement, V, may apparently be described by the relationship

$$K_C = 1.043 \sqrt{V}.$$

The closeness of fit of our data to this convection formula may be found by evaluating the radiation loss in column 8 of table 2 by use of the radiation constant found above (i.e., 3.35).[1] The convection loss is then determined by difference (column 9). The total convection constant (the rate of heat loss by convection per degree difference between the clothed surface of the body and of the air) is given in column 11.

We find, for the three series under consideration, the following mean values for K_C at their associated air movements.

SERIES	K_C	V	$\sqrt{V}$
A	2.13	4.6	2.1
B	6.25	34.0	5.8
C	16.91	264.0	16.2

[1] The result of using 3.35 with T_A is the same as if 3.59 were used with the true value of T_W (after applying the correction for reflection of body heat) and is much simpler. The value of 3.59 corresponds to normal conditions under which the body is not surrounded by reflecting walls.

Plotting these data as the solid line in figure 3, we find an excellent fit with the formula given above.

When convection constants are computed by the above procedure, it is possible for the first time to compare convection constants derived under conditions of equal-wall and equal-air with those found for cold-air and warm-wall conditions. This comparison is most instructive for the conditions without forced air movement.

In order to determine whether the cold-air hot-wall situation might exert any specific influence, we conducted a fourth series of experiments, (series D) with air at 16.2° and walls at 26.9°, with no forced air movement. The basic data (corresponding to table 1) are not presented; but the deriva-

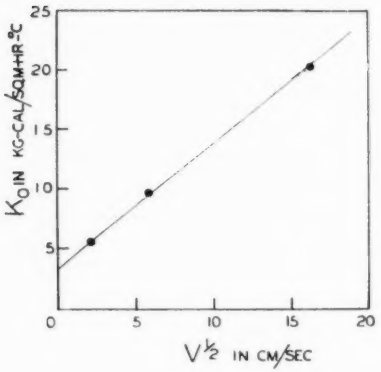


Fig. 2

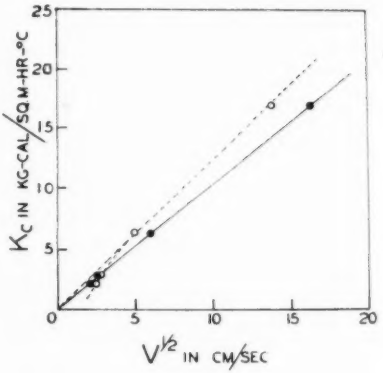


Fig. 3

Fig. 2. Relation between environmental constant (K_O) and air movement.

Fig. 3. Relation between the total convection constant (K_C) and air movement, when latter is measured without subject in booth (closed circles) and when with subject in booth (open circles).

tive data are summarized in table 3. Here a mean value of 2.75 was obtained for K_C for an unforced air movement of 6.1 cm. per second around the subject. In series A, with a similar unforced air movement of 4.6 cm. per second, the mean value for K_C was 2.13. In the papers cited above, we reported—for the same two subjects—a value of K_C of 3.24 for unforced air movement of 7.6 cm. per second (Gagge, Winslow and Herrington, 1938).

Since it is not possible to produce identical degrees of unforced air movement in different series, the measured values of air movement must be considered in determining whether or not there is a true difference in the convection constant in these three results which is due specifically to the factor of radiation. The evidence for the square root relationship,

as has been shown, is very considerable. If we approach this comparison directly on the square root assumption we may examine this question through dividing the experimental K_c values of 2.13, 2.75, and 3.24 by the square root of the respective measured air movements of 4.6, 6.1, and 7.6 cm. per second. The three dividends, 0.933, 1.112, and 1.178 are the resulting approximations of the constant before the radical in the formula $K_c = k \times \sqrt{V}$. The mean of these values is 1.094 and all three values lie within ±9 per cent of their mean. This percentage deviation is well within the experimental error of our observations on these derived constants and the conclusion may be safely drawn that the comparison of the convection constants derived for equal air and wall conditions and for cold-air and hot-walls after corrections for variations in air movement, does not indicate for our present limits of experimental error a specific effect of high radiation on the radiation constant. This comparison, however, does draw particular attention to the importance of relatively small changes in air movement when air movement and convection losses are low.

Measurement of air movement. In the experiments here reported the measurement of air movement has been made *without* the subject present in the booth. This is contrary to the procedure reported by us in our earlier papers on air movement (Winslow, Herrington and Gagge, 1938; Gagge, Winslow and Herrington, 1938). In our earlier work, with the subject in the booth, measurements were made at points corresponding to the skin or clothing temperature points and at a distance 15 cm. from the surface of his body. On further consideration of the problem, it was apparent that two sorts of air movement were involved—the primary movement produced by the exterior forces at work in the booth itself (due to normal air convection, with or without local fans) and modifications of such movement produced by the inductive influence of the warm body of the subject, or by the interference of the body of the subject with the currents set up by exterior forces.

A comparison of the observed air movement values in cm./sec. as measured by the two methods outlined in the previous paragraph is indicated in the table below:

AIR MOVEMENT	SERIES			
	A	D	B	C
Without subject	4.6	6.1	34	264
With subject	6.1	7.6	24	191

It will be noted that at low initial air movements (due to circulation produced by the normal air-conditioning apparatus of the shell-space outside the booth) the presence of the subject increased air velocity by

induced convection currents. At high initial air movements (produced by fans within the booth) the interference of the body of the subject markedly decreased air movement by physical obstruction.

In figure 3 the solid line represents relations computed on velocities measured without the presence of the subject. The dotted line shows values of the observed convection constants for the series A, B, C and D above, with the observations of air movement taken with the subject in the booth.

It is interesting to note that for the high air movement readings, even when measured with the subject in the booth, the square root relationship holds and validates general conclusions based on our earlier air movement

TABLE 3*

Series D—Elements of thermal interchange with warm walls (26.9°C.) and cool air (16.2°C.) and minimal air movement (6.1 cm./sec.)

PERIOD	M	E	ΔT_S	ΔT_R	ΔT_B	S	$\frac{M-E+S}{R+C}$ OR	$\frac{T_{Cl}}{T_W}$	R	C	$\frac{T_{Cl}}{T_A}$	k_C	$\frac{T_S}{T_{Cl}}$	K_{Cl}
minutes	*kg.-Cal./sq.m.-hr.*	*kg.-Cal./sq.m.-hr.*	*°C.*	*°C.*	*°C.*	*kg.-Cal./sq.m.-hr.*	*kg.-Cal./sq.m.-hr.*	*°C.*	*kg.-Cal./sq.m.-hr.*	*kg.-Cal./sq.m.-hr.*	*°C.*	*kg.-Cal./sq.m.-hr.-°C.*	*°C.*	*kg.-Cal./sq.m.-hr.-°C.*
20–60	45.2	11.4	0.10	0.07	0.08	4.7	38.5	1.4	5.5	33.0	12.15	2.7	5.22	7.4
40–80	44.2	11.4	0.14	0.08	0.10	6.0	38.8	1.4	5.5	33.3	12.15	2.7	5.11	7.6
60–100	43.3	11.4	0.17	0.06	0.09	5.7	37.6	1.4	3.2	34.4	12.10	2.8	5.05	7.4
80–120	43.4	11.4	0.20	0.05	0.10	6.0	38.0	1.3	2.9	35.1	11.99	2.9	4.94	7.7
100–140	43.5	11.4	0.18	0.04	0.09	5.4	37.5	1.2	4.6	32.9	11.88	2.8	4.88	7.7
120–160	43.2	11.4	0.14	0.05	0.08	5.0	36.8	1.2	4.6	32.2	11.93	2.7	4.88	7.5
140–180	43.0	11.4	0.14	0.04	0.08	4.7	36.3	1.2	4.6	31.7	11.93	2.7	4.83	7.5
160–200	43.7	11.4	0.15	0.02	0.06	3.7	36.0	1.1	4.2	31.8	11.82	2.7	4.77	7.5
												m2.75		m7.54
Col.	1	2	3	4	5	6	7		8	9	10	11	12	13

* For explanation see text.

work. At low air movements, however, the line on the graph plotted from air measurements adjacent to the body of the subject departs from linearity and does not approach the origin as it must logically do. Therefore, the measurement of air movement without the presence of the subject is clearly indicated as both practically and theoretically the correct procedure.

Since no means other than empirical formulae have ever been found for describing convection processes, it is quite possible to develop a useful empirical formula in terms of values obtained by any fixed manner of measurement. It seems clear, however, that the analytical situation is simpler when the air velocity is measured in the empty booth before the entrance of the subject; and that such a procedure yields clean-cut and

consistent results. For once accuracy and technical convenience are served by the same method, for it is certain that wide application can be made, both in the laboratory and in practical situations, of air movement data which do not require numerous local measurements about the body surface.

Clothing conductance. One minor point remains to be mentioned. The more accurate measurements of T_s and T_{cl}, discussed in an earlier paragraph, make possible a much better estimate of the conductance of the clothing than that presented in our earlier paper (Gagge, Winslow and Herrington, 1938). In columns 12 and 13 of tables 2 and 3 are given the values for $T_s - T_{cl}$ and the resulting K derived by dividing $R + C$ by $T_s - T_{cl}$. For series A, series C and series D, this value, in each case, averages about 7.5 to 7.6, while for series B it is slightly lower, 6.6. An average figure of about 7.3 for the clothing conductance is, therefore, indicated, which is materially lower than the value reported in our earlier work. The effects of the higher T_s values and the lower T_{cl} values obtained by the present more accurate techniques are, of course, additive in this computation.

SUMMARY OF CONCLUSIONS

1. Four series of experiments are reported in which two young male subjects, lightly clothed and in a semi-reclining position, were observed for periods of 200 minutes in cool environments. The cooling effect was such as to cause storage (chilling of body tissues) at a rate of 3 to 14 kilogram-calories per square meter of body surface per hour. Air and wall temperatures and air movement were the experimental variables employed. Additional precautions in the construction and use of thermocouples and thermopiles made possible more accurate records of skin and clothing temperatures than those previously reported by us.

2. Under the conditions of these experiments we found (as did Berkson and Boothby under similar circumstances) that metabolism falls by about 5 per cent during the first two hours (probably due to relaxation) and then rises again (perhaps as a result of restlessness).

3. Under such moderately cool conditions as those here employed, the skin temperature of the trunk falls only slightly and reaches a stable level after one hour and a quarter. The head temperature falls more rapidly but stabilizes after two hours. The skin temperature of the upper extremities falls very sharply and continues to fall for 200 minutes, though less rapidly after the first hour. The skin temperature of the lower extremities continues to fall steadily and at almost the same rate for the whole 200-minute period, and so does the rectal temperature, although its fall is, of course, much less rapid. During the whole course of such an experiment rectal temperature drops about a quarter of a degree; skin temperature

of the trunk about 0.5°; of the head, 1°; of the lower extremities, 2°; and of the upper extremities, 3°.

4. Storage has been estimated by assuming that actual change in tissue temperature is influenced by rectal temperature changes and skin temperature changes respectively, in the ratio of 2:1. This assumption, which is essentially that of Burton, appears to be validated (for the conditions of our experiments and his) by the general concordance of results obtained. For a warmer environment, the 4:1 ratio of Hardy and DuBois might well obtain.

5. The radiation area of our two subjects was estimated in earlier studies by varying wall temperatures and keeping other factors constant. In the present experiments we determined this factor by varying air movement and plotting the constant for radiation plus convection against a power of air velocity which yielded a linear relationship—the ordinate intercept giving the radiation constant. After correcting this radiation constant by a factor, which measures the reflection of radiant body heat from the copper walls, the present method gave a radiation area equal to 71.5 per cent of the DuBois area, as compared with 75.5 per cent obtained by the earlier procedure. We are inclined to believe that the lower area now observed may represent a real difference due to an attempt to secure protection against chilling by closer apposition of the limbs.

6. We have been led by logical analysis of our data to conclude that in evaluating the cooling effect of air movement the air velocity can best be measured in the area to be occupied by the subject before the entrance of the subject and at such a distance from the observer as not to be influenced by his body. Low initial air movements are increased by induced air currents due to the warmth of the body. High initial air movements are reduced by the physical obstruction of the body. The use of air velocities actually observed near the body, as influenced by these processes, yields results which are less consistent than those obtained from computations based on initial air velocities unaffected by the subject himself.

7. When the above methods are employed, it is found that the cooling effect of air movement varies with square root of velocity.

REFERENCES

Bazett, H. C. and B. McGlone. This Journal **82:** 415, 1927.

Berkson, J. and W. M. Boothby. This Journal **121:** 669, 1938.

Burton, A. C. J. Nutrition **9:** 261, 1935.

Hardy, J. D. and E. R. DuBois. J. Nutrition **15:** 477, 1938.

Gagge, A. P. This Journal **116:** 656, 1936.

Gagge, A. P., L. P. Herrington and C.-E. A. Winslow. Am. J. Hyg. **26:** 84, 1937.

Gagge, A. P., C.-E. A. Winslow and L. P. Herrington. This Journal **124:** 30, 1938.

Winslow, C.-E. A., L. P. Herrington and A. P. Gagge. This Journal **116:** 669, 1936.

This Journal **124:** 51, 1938.

EXPERIMENTS UPON THE RELATION BETWEEN THE PITUITARY GLAND AND WATER DIURESIS[1]

ARTHUR M. WALKER

From the Laboratory of Pharmacology, University of Pennsylvania, Philadelphia

Received for publication July 18, 1939

The theory of a control over urine volume by the posterior lobe of the pituitary gland, originally advanced by Motzfeldt and elaborated by the work of Verney and his collaborators, is widely adopted as an explanation of variations in water excretion which occur under a variety of circumstances—water diuresis, diuresis from hypnotic suggestion, conditioned reflex or prolonged rest, oliguria from exercise, anesthesia, afferent nerve stimulation, acetylcholine injection, etc. (1–14). As stated by Verney, it postulates that under normal conditions the reabsorption of water in the kidney is subject to constant stimulation by the antidiuretic hormone, the degree of which is proportional to its concentration in the blood and hence to the rate of pituitary secretion, this secretion in turn being governed by nervous stimuli or by changes in the composition of the blood which are transmitted to the gland through the mediation of the central nervous system. Although this theory is in harmony with a large volume of experimental evidence there is scant proof of the presence of an antidiuretic hormone in body fluids in concentrations which vary with physiological variations in urine volume. Our experiments were designed—and have failed—to obtain this proof which seems so essential to the acceptance of the theory.

They have been confined to an investigation of water diuresis, starting with an assay of the antidiuretic potency of blood and cerebrospinal fluid specimens collected before and after the oral administration of water. Soon after they began, the paper by Gilman and Goodman (15) appeared which seemed to contain straightforward proof that the urine of rats contains the pituitary antidiuretic hormone in amounts roughly proportional to the degree of the animals' dehydration. Their results appeared so conclusive that we turned to the study of urine, hoping to confirm and perhaps to amplify them. Using their methods we encountered puzzling discrepancies between our results and theirs and became convinced of the necessity of finding a way of separating the antidiuretic substance from

[1] The expenses of this investigation were defrayed in large part by a grant from the Commonwealth Fund.

urine. We have developed a method of doing this with some degree of quantitative reliability, and it has become possible to assay its activity in the absence of most if not all other urinary constituents. Study of the occurrence of this separable antidiuretic substance did not serve to diminish the earlier discrepancies and, in the case of animals other than rats, convinced us that it is neither peculiar to the urine of dehydrated animals nor derived from the posterior lobe of the pituitary gland.

METHODS. *Assay.* Two methods have been used: the rat method of Burn (16) and one which employed diuretic rabbits. Concerning the rat method it is only necessary to say that the male albino rats used were from a single stock colony, weighed 200 to 300 grams, were kept on a uniform diet, and received 5 cc. of water per 100 grams of body weight by stomach tube immediately before injection of the preparation to be assayed. The volume of the injected dose was 1 cc. per 100 grams, its site usually subcutaneous, sometimes intraperitoneal. A group of 4 rats was used in each assay. Urine was measured at 15-minute intervals, the time required for excretion of 50 per cent of the administered water being recorded as the end point. The animals were frequently standardized both by water alone and by injections of appropriate dilutions of pituitrin.[2] The average time required for 50 per cent water excretion in control experiments was 75 minutes; in only 3 out of 163 tests did it exceed 100 minutes. It is therefore safe to interpret a figure exceeding 100 minutes as indicating a positive result and this safety forms the chief advantage of the method. It is not as accurate a method of assay as the averaged curves of figure 1 imply, and it has the disadvantage of being unable to detect the antidiuretic effect of much less than 40 milliunits of pituitrin (4 MU per 100 grams to each of 4 rats) or of distinguishing between amounts much in excess of 120 milliunits unless preliminary dilutions are made.

The rabbit assay. Male rabbits were kept on a diet which included green vegetables and were given 100 cc. of water by stomach tube several hours before the experiment. At the beginning of the experiment they were given by stomach tube a quieting dose of paraldehyde, 1 cc. per kgm., and water, 50 cc. per kgm. Urine was collected by an inlying catheter, its volume recorded every 10 minutes. Diuresis at a rate of 0.5 to 1.0 cc. per minute was usually established within 90 minutes and was maintained by subsequent administration of paraldehyde and water as necessary. The fluids to be assayed were usually injected intravenously, sometimes intraperitoneally or intramuscularly. The preparation is approximately 100 times as sensitive as is the rat method, usually reacting to as little as 0.2 and always to 0.5 MU or more of pituitrin (fig. 2, *A*, *B* and *C*). This sensitivity has made it possible, in 5 experiments, to demonstrate antidiuretic activity in the blood (fig. 2, *D*) of rabbits in which antidiuresis has

[2] Parke, Davis and Co., 1 cc. = 10,000 milliunits (MU).

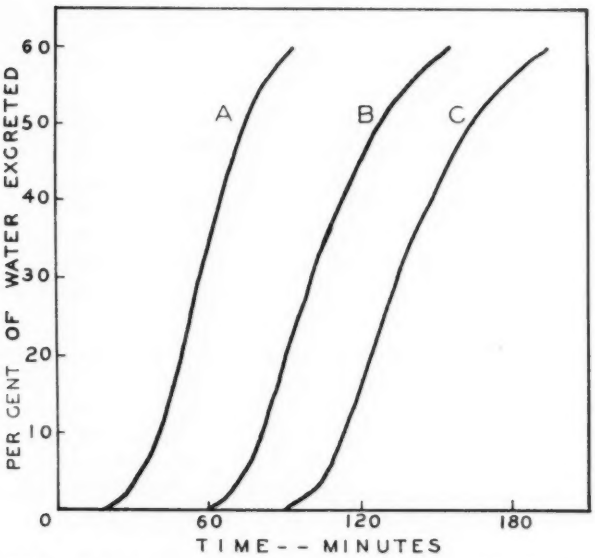


Fig. 1. Effect of pituitrin on water diuresis in rats. In addition to 5 cc. of water per 100 grams of body weight by mouth each rat was injected subcutaneously with: control curve A (average of 163 expts.) 0.9 per cent NaCl, 1 cc. per 100 grams; curve B (average of 35 expts.) 4 MU pituitrin per 100 grams; curve C (average of 31 expts.) 8 MU pituitrin per 100 grams.

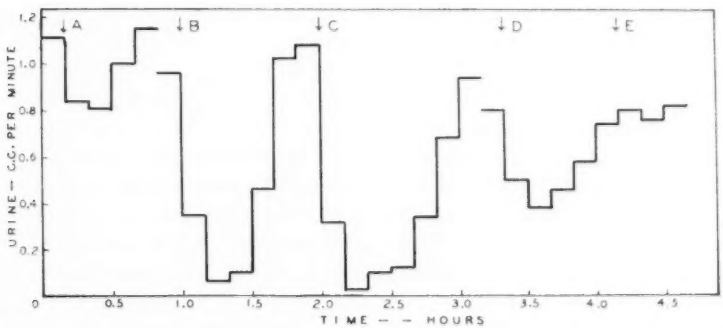


Fig. 2. Effect of pituitrin on water diuresis in rabbits. Typical effects of intravenous injection, into the same diuretic rabbit, of: A - 0.5 MU pituitrin, B - 2.0 MU pituitrin, C—5.0 MU pituitrin, E—2.0 cc. 0.9 per cent NaCl solution. At D, 5 cc. of whole blood was injected from a donor rabbit which had received 2000 MU pituitrin subcutaneously 60 minutes previously.

been produced by subcutaneous injection of 1 to 2 units of pituitrin 60 minutes before the transfusion; the amounts thus recovered were small and sustain the conclusion (17) that injected pituitrin disappears very rapidly from the circulating blood.

The preparation has two disadvantages as a method of assay. Such differences in sensitivity are encountered in different rabbits or in the same rabbit from day to day that it is necessary to test injections of known concentrations of pituitrin in sequence with the unknown preparation in order to obtain quantitative results. Despite the use of paraldehyde, interruptions in diuresis occur spontaneously or from struggling with sufficient frequency so that great caution is necessary in accepting individual results.

Recovery of antidiuretic substance from urine. Various commonly used adsorbents remove the antidiuretic principle of pituitrin from saline and, less successfully, from urine but in our experience do not allow its satisfactory recovery by elution. One exception to this statement is collodion. When a solution of pituitrin at a pH of 6.0 to 8.0 is filtered through a collodion membrane most of it is adsorbed by the membrane and can be released by the subsequent filtration of N/5 HCl.

Test tubes, 12 mm. internal diameter, were filled with a 7 per cent solution of parlodion in alcohol-ether (60:40), emptied, rotated for two minutes and fixed in an inverted position for 30 minutes. After removal, the sacs were kept in distilled water for at least 24 hours before use and were tested to withstand a pressure of 150 mm. Hg. The urine specimen, reduced in volume to 20–40 cc. when necessary by evaporation at reduced pressure and temperatures below 40°C., was adjusted to a pH of 6.0–8.0 and filtered through paper. It was then filtered through the collodion sac at a pressure of 140 mm. Hg until the filtrate amounted to 25–50 per cent of the urine (1–3 hrs.). The unfiltered residue was removed and the sac washed by rinsing both surfaces with distilled water and by filtering distilled water through it for 5 minutes. N/5 HCl was then slowly (20 min.) filtered through the sac until enough fluid for assay had been recovered (5–10 cc.). The colorless and odorless acid filtrate was neutralized with NaOH to a pH of 7.0 to 7.6 and its antidiuretic potency determined by the rat or rabbit method. The pituitrin equivalence of the entire specimen was calculated on the basis of the fraction which had been ultrafiltered. Cerebrospinal fluid was similarly handled; its filtration was more rapid, more complete, and the preliminary reduction in volume was unnecessary.

Table 1 demonstrates that the adsorption method recovered, on the average, 81 per cent of the antidiuretic principle of pituitrin which had been added to the urine of man, rabbits, and rats in concentrations of 1 to 30 MU per cc. The variations in recovery are largely due to inaccuracies of assay; in part also to the fact that, when added to urine, a fraction (10–

20 per cent) of the antidiuretic principle escapes adsorption and appears in the ultrafiltrate. This fraction increases at pH below 6.0 until, at pH below 3.0, all of the antidiuretic principle escapes adsorption.

ANTIDIURETIC ACTIVITY OF RABBITS' BLOOD. In 27 experiments 5 cc. of whole blood was taken by cardiac puncture from rabbits which had been made diuretic by oral administration of water (average urine flow, 0.41 cc. per min.) and immediately injected intravenously into diuretic rabbits prepared for assay; no anticoagulant was used. In no case was the injection followed by antidiuresis (fig. 3*A*).

TABLE 1

Recovery from urine of antidiuretic fraction of pituitrin by collodion adsorption method

ADDED MILLIUNITS	RECOVERED	
	Milliunits	Percentage
5	5	100
10	5	50
40	20	50
61	55	90
90	104	116
90	61	68
100	72	72
105	132	126
124	108	87
133	83	63
143	96	67
144	98	69
146	105	72
148	112	77
184	135	74
210	243	116
330	240	73
Average		81

Ninety-five similar transfusions[3] were made from rabbits which had been without food or water for from 18 to 120 hours (average urine flow 0.027 cc. per min.). In 32 cases slight or moderate antidiuresis occurred; 17 of these were open to suspicion because of a slight struggle by the recipient or because the antidiuresis developed after a latent period of more than 10

[3] In 11 additional experiments the blood was injected intraperitoneally. Definite antidiuresis occurred in 9, regardless of whether the animal which supplied the blood was hydrated or dehydrated. Similar injections of 0.9 per cent sodium chloride solution produced no such effect (30 tests). The action of pituitrin, injected intraperitoneally, is not much more prolonged than when given intravenously. We offer no explanation for these results.

minutes. This leaves 15 results (fig. 3, *B* and *C*) which indicate that the blood of dehydrated rabbits, in contrast to hydrated, may contain something which is antidiuretic, but the difference was not sufficiently constant to warrant any attempt to identify the nature or source of the substance present. The incidence of positive results was not increased by transfusing more than 5 cc. of blood, and in several cases a donor which yielded blood with antidiuretic activity on one occasion failed to do so after further dehydration.

We cannot regard these results as providing adequate support for the experiments of Marx (18) who described an antidiuretic substance, believed to be of pituitary origin, in the blood of dehydrated dogs. Substances with antidiuretic effects have been repeatedly described in the blood of humans

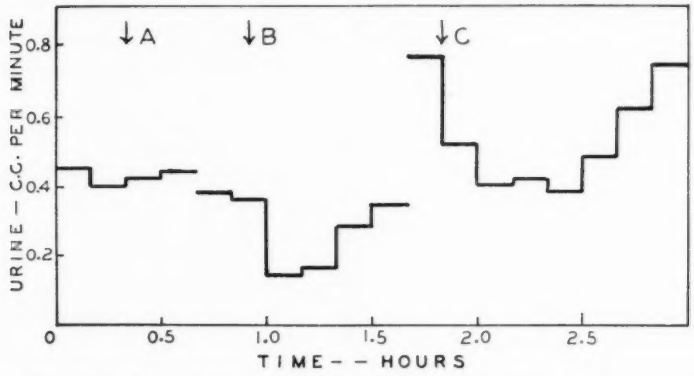


Fig. 3. Effect of rabbits' blood on water diuresis in rabbits. Intravenous injection of 5 cc. whole blood from: A—diuretic rabbit 9, urine 1.0 cc./min. 11/5/36. B—24-hour dehydrated rabbit 9, urine 0.01 cc./min. 11/5/36. C—120-hour dehydrated rabbit 40, urine 0.009 cc./min. 12/31/36.

(19–22), dogs (20), and rabbits (23) but there have been no other attempts to associate the presence of these substances with alterations in urine volume and indeed there seems no particular reason to identify them with the antidiuretic principle of the pituitary gland (22, 23).

Antidiuretic Activity of Cerebrospinal Fluid. In 7 out of 19 trials spinal fluid taken by cisternal puncture from unanesthetized rabbits, and in 1 trial fluid collected by lumbar puncture from a normal unanesthetized man, produced prompt slight antidiuresis when tested by the rabbit method (fig. 4*A*). Similar fluid was collected from 8 dogs by cisternal puncture with or without the preliminary subcutaneous injection of morphine sulphate, and separation of an anti-diuretic substance was attempted by the collodion adsorption method. All of the dogs had free access to water for

the preceding 24 hours. Positive results (fig. 4*B* and *C*), obtained in 5 instances, were such as would have been produced had the cerebrospinal fluid contained approximately a 1:50,000 dilution of pituitrin. Since 2 of the dogs had been subjected to total pituitarectomy the substance apparently[4] does not originate in the posterior lobe of the pituitary gland. Since all the dogs were hydrated the substance is certainly not peculiar to the cerebrospinal fluid of dehydrated animals. The experiments do not reveal whether or not the concentration of the substance varies when variations in water intake are imposed. This record is made because, in so far as we are aware, the observation of an antidiuretic constituent of cerebrospinal fluid has not previously been presented.

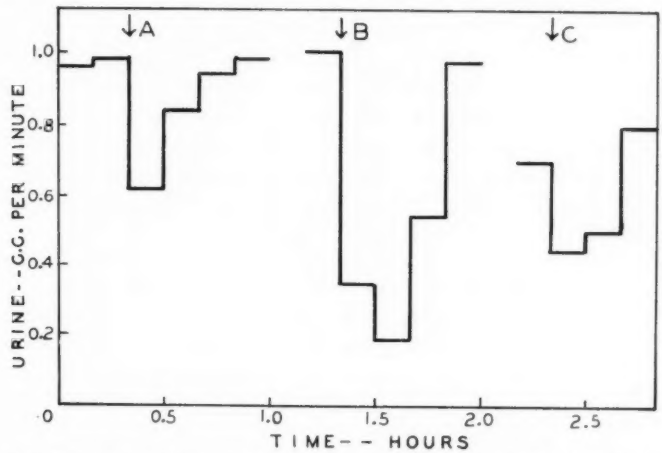


Fig. 4. Effect of cerebrospinal fluid on water diuresis in rabbits. Intravenous injection of: A—1.0 cc. unaltered cerebrospinal fluid from a 48-hour dehydrated unanesthetized rabbit. B—Extract of 5 cc. cerebrospinal fluid from hydrated dog; morphine sulphate. C—Extract of 8.5 cc. cerebrospinal fluid from pituitarectomized, hydrated, unanesthetized dog.

ANTIDIURETIC ACTIVITY OF URINE. *Cats.* In 1937, 12 specimens of urine were collected from 8 normal cats, dialyzed 3 hours against 0.9 per cent sodium chloride solution through no. 300 cellophane (0.00088 in.),[5] and tested

[4] The fluids were obtained by the courtesy of Dr. R. C. de Bodo from animals which had been operated upon by Dr. J. E. Sweet of New York. Since autopsy findings will not be available for several months the results of these assays, extended to a larger series, will be made the subject of a subsequent note.

[5] This cellophane had been in the laboratory since 1936 and was much more permeable than that, with the same stated wall thickness, which is now available. Unfortunately, the success of the dialysis was not routinely observed but in 4 instances initial urea nitrogen concentrations of 0.7 to 3.8 per cent decreased to less than 0.1 per cent.

without previous evaporation by the rabbit method. The specimens were injected on 36 occasions by intravenous, intraperitoneal and intramuscular routes in amounts equivalent to 1/48 to 1/24 of the 24-hour output. Four of the animals were then subjected to complete pituitarectomy by Dr. F. D. W. Lukens and 9 specimens, taken 2 to 12 days after operation, were assayed by 36 injections. Urine from all but 1 of the animals produced antidiuresis and it was clear that dehydration did not exaggerate nor pituitarectomy diminish the magnitude of the response.

The conclusion was confirmed by the results of a later series of experiments in which the antidiuretic constituent was separated from the urine of cats, hydrated and dehydrated, normal and pituitarectomized. Fifteen 24-hour specimens were collected from 5 cats: 3 of the 5 were then sub-

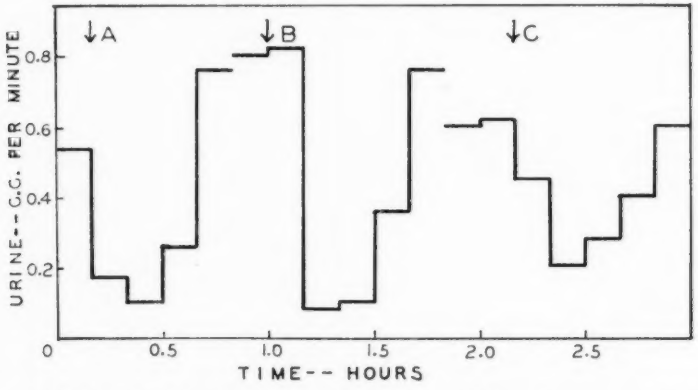


Fig. 5. Effect of cats' urine on water diuresis in rabbits. Intravenous injection of: A—Extract of urine from hydrated cat 23; 24-hour volume 182 cc. B—extract of urine from 72-hour dehydrated cat 23; 24-hour volume 24 cc. C—extract of urine from cat 23, 4 days after total pituitarectomy; 24-hour volume 54 cc.

jected to complete pituitarectomy by Dr. F. D. W. Lukens[6] and, from these, 15 additional specimens were obtained from 1 to 11 days following operation. The 24-hour volumes varied from 440 to 18 cc., specific gravities from 1012 to 1057, according to the degree of hydration or dehydration imposed. The pH range was from 5.8 to 6.9. From 25 to 63 per cent of each specimen was filtered through collodion and the entire acid elution assayed by the rabbit method. The results obtained are represented in figure 5 and can be summarized as follows:

1. There is a small amount of antidiuretic substance in the urine of cats

[6] One pituitary fossa was entirely clear at autopsy; the other two contained small fragments of tissue which, upon section and examination by Dr. Balduin Lucké, were found to consist entirely of necrotic fibrous tissue and necrotic leucocytes.

which have free access to water. This was true of 5 out of 7 specimens from normal cats with 24-hour urine volumes of from 92 to 210 cc. In terms of pituitrin, the amounts were of the order of from 2 to 20 MU per cat per day.

2. The amount of antidiuretic substance in the urine of normal cats is sometimes increased by withholding water for from 24 to 72 hours but in an equal number of instances it is not increased by this procedure (fig. 5, *A* and *B*). None of the 8 specimens of this series was free from antidiuretic substance.

3. The presence of the pituitary gland is not essential for the occurrence of antidiuretic substance in the urine for it was found in essentially unaltered amounts in postoperative specimens from each of the 3 animals (fig. 5, *C*). It is true, however, that in 6 out of 15 postoperative specimens no antidiuretic substance was extractable and in 2 of the operated animals it appeared in the urine only after the transient polyuria following the operation had subsided spontaneously or as a result of withholding water.

An item of possible significance in connection with the identity of this antidiuretic substance and the pituitary hormone appeared in 9 of the 22 positive injections made in this series as well as in a number of the experiments with cerebrospinal fluid and rabbits' urine, viz: that a latent period of 10 minutes preceded the onset of the antidiuresis (fig. 5*B*) whereas, in our experience, when pituitrin was injected the latent period never exceeded 3 minutes. A latent period of 10 minutes has, however, been commonly observed with the antidiuresis produced by extracts of lung and liver (heparin) (24).

These experiments indicate the presence in cat urine of small amounts of an antidiuretic substance which is not derived from the posterior lobe of the pituitary gland nor increased in amount by dehydration. They are at variance with the preliminary report of Ingram, Ladd, and Benbow (25) who found no antidiuretic substance whatever in the urine of hydrated animals and very much larger amounts than we have described after "strenuous" dehydration.

Rabbits. In a group of experiments made in 1937, 38 specimens of urine representing 10 to 30 minute excretion periods were collected by catheter from 15 hydrated and dehydrated rabbits. Either without treatment or after dialysis for 3 hours against 0.9 per cent sodium chloride solution through no. 300 cellophane, they were tested for antidiuretic activity by intravenous or intraperitoneal injection into diuretic rabbits. Thirty-seven of the 49 injections were followed by marked antidiuresis. When the dosage of urine from dehydrated and hydrated rabbits was adjusted to represent equal periods of time of excretion, it was apparent that the degree of dehydration of the donor rabbit was not an important factor in the magnitude of the antidiuresis.

This opinion was confirmed by a later series of experiments in which the antidiuretic substance was separated from urine. Twenty-four-hour specimens were collected, 25 to 50 per cent of each filtered through collodion, the adsorbed antidiuretic factor eluted and its solution injected intravenously into diuretic rabbits. Thirteen specimens were from 5 rabbits, hydrated by repeated injections of water by stomach tube, and averaged 235 cc. for 24 hours with a specific gravity of 1011. Seven were from the same rabbits dehydrated as long as 72 hours and averaged 34 cc. for 24 hours with a specific gravity of 1042. No experiments upon pituitarectomized rabbits have been made.

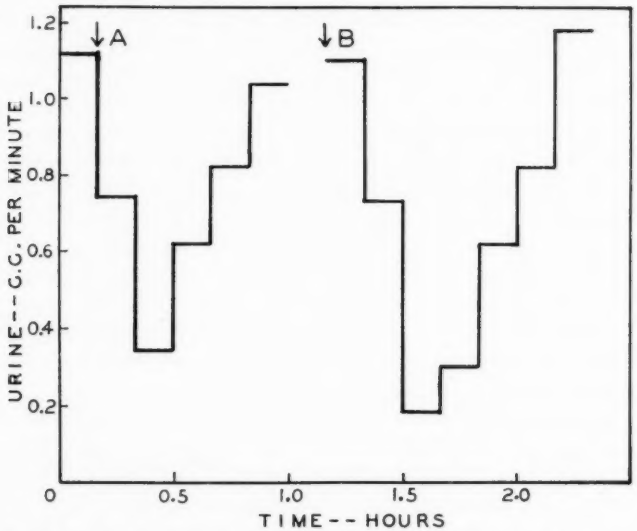


Fig. 6. Effect of rabbits' urine on water diuresis in rabbits. Intravenous injection of: A—extract of urine of hydrated rabbit 4; 24-hour volume 275 cc. B—extract of urine of 72-hour dehydrated rabbit 4; 24-hour volume 41 cc.

The urine of one animal contained no antidiuretic substance at any stage of hydration or dehydration. Of the others, all but 3 of the hydrated and all of the dehydrated specimens contained antidiuretic substance, the majority of comparisons of urines from the same animal indicating a somewhat greater amount in the dehydrated specimen (fig. 6, *A* and *B*). The difference has seemed neither great enough nor constant enough to represent an important factor in water diuresis; indeed the amounts which we have recovered are so small—in no instance either in rabbits or cats exceeding the equivalent of 20 MU of pituitrin per animal per day—as to make us skeptical of its physiological importance.

Dogs. Nine urine specimens from 2 dogs have been studied by the collodion adsorption method. Specimens from one hydrated animal, averaging 600 cc. for 24 hours, yielded antidiuretic substance equivalent to 10–25 MU of pituitrin. Scarcely more than that was obtained after the animal had been without food and water for 24 hours. Urine specimens from the second animal, when hydrated, yielded no recoverable antidiuretic substance and only the equivalent of 8 MU per 24 hours after 48 hours of fasting and dehydration. No urine specimens from pituitarectomized dogs were examined. The results of this small series seem to be in accord with those performed on cats and rabbits.

Rats. Gilman and Goodman (15, 26) have reported finding considerable amounts of an antidiuretic substance in the urine of dehydrated rats; the absence of this substance from the urine of hydrated rats or of dehydrated pituitarectomized rats has led them to conclude that this substance is secreted by the posterior pituitary body and is responsible for the oliguria which dehydration produces. Our failure to make similar observations on other animals led us to repeat their experiments upon rats according to their procedure, to use various modifications of it, and to apply the collodion adsorption method described on page 522.

In a series of 25 experiments upon dehydrated animals we have adhered to Gilman and Goodman's procedure. Groups of 12 to 24 rats were deprived of water for from 24 to 96 hours; 24-hour urine volumes fell as low as 1.0 cc. per animal; specific gravity of urines rose as high as 1065; their pH was maintained between 5.6 and 6.3 by the addition of 1 per cent acetic acid to the collecting vessels. Each specimen was evaporated, when necessary, under reduced pressure and at temperatures below 40°C. to from 10 to 20 cc., dialyzed for 3 hours against running tap water in cellophane sacs (wall thickness, 0.00072 in.) till its specific gravity fell below 1010, and was injected subcutaneously in whole or in part for assay by the rat method. Three such specimens collected during the first 24 hours of dehydration showed no antidiuretic activity. Of the others, collected after longer dehydration, only 3 failed to produce antidiuresis. As Gilman and Goodman found, the antidiuretic potency did not increase with longer dehydration than 48 hours. In terms of pituitrin, it never exceeded 20 MU per rat per day and averaged 9 MU. In 3 of 4 similar experiments, performed upon rats having free access to water, the urine showed no antidiuretic activity; in the fourth the result indicated the excretion of antidiuretic substance equivalent to 2 MU of pituitrin per rat per day.

The chief results of these experiments were confirmed by 3 series of tests in which the antidiuretic substance was separated by the collodion adsorption method. The results were consistent and may be illustrated by a single series.

DATE	VOLUME OF URINE	SPECIFIC GRAVITY	DEHYDRATED	MU ANTIDIURETIC SUBSTANCE PER RAT
	cc. per rat		*hours*	
12/ 7/38	5.7	1021	0	2.5
12/ 8/38	3.1	1026	0	0.0
12/ 9/38	1.6	1052	24	5.5
12/10/38	1.7	1065	48	9.0

These results show clearly enough that the urine of rats deprived of water contains an antidiuretic substance which is not consistently present in urine of rats on normal water intake. This constitutes confirmation of Gilman and Goodman's findings, though the amounts which we discover are considerably less than theirs and more nearly resemble those found in the confirmatory experiments of Boylston and Ivy (27).

In addition to the experiments with rats summarized above a good many others have been made which indicate that the matter is not as simple as those results indicate. They also throw light on the question of identity of the antidiuretic substance in urine with that in commercial postpituitary extracts. They are discussed in the following 5 sections.

1. The absence of antidiuretic substance from the urine of dehydrated pituitarectomized rats, described by Gilman and Goodman, is the chief reason for believing that this substance is derived from the pituitary gland. We have confirmed their observation in 4 experiments but attach less importance to it than do they; partly because gross changes in other glands of internal secretion follow complete pituitarectomy; partly because operated rats are so susceptible to water deprivation that they are usually moribund after 48 hours. We have also confirmed Gilman and Goodman's observation that the antidiuretic substance of urine, like that of pituitrin, is destroyed by 30-minute hydrolysis with 2 N NaOH and have observed several other similarities between the two: notably their adsorption on activated charcoal, collodion, and Lloyd's reagent, their resistance to boiling, their filtrability and dialyzability through cellophane membranes of various thicknesses. But in one particular, that of their stability in rat urine, the antidiuretic substance of urine behaves so differently from that of pituitrin as to warrant the conclusion that they are not identical.

When pituitrin was added to rats' urine to make concentrations of from 1:800 to 1:2500 and allowed to stand for 24 hours at room temperature its antidiuretic activity was found to decrease when the pH of the urine was above 4.2 and to disappear above 6.6. Table 2 summarizes the data from 41 tests of this kind.

Similar results were obtained when the temperature of the urine mixture was kept at 0°C. and when the urine was boiled before adding the pituitrin. A fraction of the pituitrin disappears within the first hour but can be made

to reappear by boiling the mixture or by acidification. After 24 hours neither of these means restores the antidiuretic activity.

Were the antidiuretic substance of dehydrated urine identical with that in pituitrin one would anticipate that the amount found in the urine would be increased by allowing the urine to collect in stronger acid. In 2 series of experiments 36 per cent acetic acid was alternated with 1 per cent in the collecting vessels but no significant alteration in the result was observed. A more precise experiment was tried in 3 instances in which a pooled specimen of urine from dehydrated animals was divided into two portions, the pH of one raised to 7.1–7.5, of the other lowered to 2.8–3.0, and both allowed to stand for 24 hours. The average end point of the alkaline mixture was 117 minutes, of the acid, 118. In parallel experiments in which pituitrin was added to urine, the mixture kept at pH 7.2 gave no anti-

TABLE 2

Stability of antidiuretic fraction of pituitrin in rat urine

NUMBER OF EXPERIMENTS	pH OF URINE	AVERAGE TIME REQUIRED FOR 50% WATER EXCRETION AFTER INJECTION (1 CC./100 GRAMS) OF URINE WHICH ORIGINALLY CONTAINED PER CC.:		
		4 MU	8 MU	12 MU
		minutes	*minutes*	*minutes*
16	6.6–7.4	97	86	74
8	5.0–5.8		103	130
9	4.2–4.9	120	141	
8	2.6–4.2	131	162	194
79 controls with freshly prepared pituitrin in 0.9% sodium chloride solution		133	163	186

diuresis (73 min.), whereas that kept at 2.8 delayed 50 per cent excretion to 179 minutes.

These results indicate complete stability of the antidiuretic substance of urine in the pH range of 2.8–7.5 and the complete destruction of the antidiuretic fraction of pituitrin at the higher pH. This difference is incompatible with identity.

2. We have studied the capacity of rats to excrete injected pituitrin since this capacity would seem to be a prerequisite to identifying the antidiuretic substance of urine with a pituitary secretion. In a series of 10 experiments, groups of from 3 to 15 rats have been injected subcutaneously with single or repeated (4 to 12) doses of pituitrin in amounts totalling between 400 and 28,000 MU. The urine of the ensuing 4 to 24 hours was collected at a pH usually below 4.6 and was assayed either after dialysis through thick cellophane membranes or after extraction by the collodion adsorption method. In 8 of the 10 experiments no antidiuretic activity

of urine, attributable to the injected pituitrin, could be demonstrated despite the fact that the animals were clearly under its influence. In one experiment, for example, in which the normal water intake was simulated by hourly administration of water, several of the animals developed convulsions and died. In the 2 experiments in which injected pituitrin appeared to be excreted in the urine each animal received a single dose of 1000 MU and eliminated only 28 MU.

It would appear from this that the rat only excretes pituitrin in the urine when concentrations far higher than those necessary to produce oliguria are present in its tissues; in this respect it differs from the rabbit, for the intravenous injection of as little as 5 MU in a rabbit was followed by its appearance in the urine (see also Heller, 28). These experiments upon rats are similar to those described by Gilman and Goodman (26); in order to reconcile this finding with their belief that the antidiuretic substance in rat urine was derived from the pituitary gland they were driven to the suggestion that "the body handles the foreign pharmaceutical preparation differently than the natural antidiuretic hormone." This is, of course, a possible interpretation but until the appearance of evidence in its favor the more obvious interpretation is one which denies identity between the antidiuretic substance and a pituitary secretion.

3. In the technique prescribed by Gilman and Goodman, 0.00072 inch cellophane was used for dialysis with the expressed conviction that pituitrin was retained within such membranes during 3 hours of dialysis against running tap water. This has not coincided with our experience for in 10 experiments a large fraction (averaging over 50 per cent) of the antidiuretic principle has escaped through the membrane from both saline and rat urine and, in 2 experiments, its presence in the dialysate has been demonstrated. Reasoning that, if the antidiuretic substance in dehydrated rat urine were identical with the antidiuretic fraction of pituitrin its concentration should be increased by altering the technique of dialysis, we have performed 36 experiments in which the period of dialysis or the amount of water against which the urine was dialyzed was reduced or thicker cellophane membranes (0.00088 or 0.0034) inch were used. Despite the fact that these thicker membranes notably retarded the dialysis of sodium chloride and presumably would arrest the escape of pituitrin, no increase in the recovery of antidiuretic substance was observed. The maximum antidiuretic effect encountered per rat per day was the equivalent of 23 MU of pituitrin, the average of all, 11 MU—values scarcely greater than those observed when the procedures of Gilman and Goodman were accurately followed. Nor was any difference observed when, in 3 experiments, the urine was divided into two parts, one being dialyzed through 0.00072 inch and the other through 0.0034 inch. It was because of these inconsistencies in the results of the dialysis technique that it

seemed essential to discover a way of separating the antidiuretic substance from urine and as a result the collodion sac method was developed. Its application to dehydrated urines, however, instead of increasing the yield as our control experiments gave us the right to expect, if anything decreased it. Seven experiments on a group of 24 rats dehydrated for from 24 to 72 hours gave an average recovery of only 5.2 MU per rat per day, the maximum recovery in any single experiment being 10 MU.

Employing the modified dialysis technique, with membranes thicker than 0.00072 inch, 35 specimens of urine from *hydrated* animals were assayed by the rat method. In 25 of these experiments, marked antidiuretic effects were observed. The curve of water excretion, however, differed from

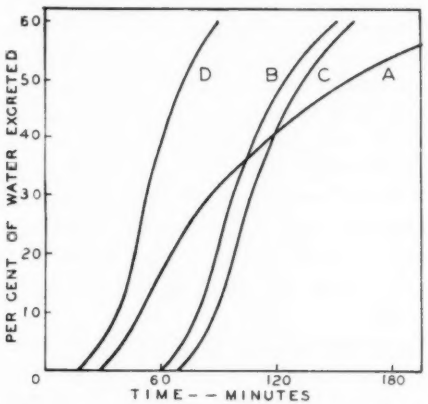


Fig. 7. Effect of rats' urine on water diuresis in rats. Typical effects of injecting: A—subcutaneously, urine from hydrated rats, normal or pituitarectomized, after dialysis in thick cellophane (0.0034 in.). B—subcutaneously, pituitrin 4 MU per 100 grams. C—intraperitoneally, urine from hydrated animals—normal or pituitarectomized, after dialysis in thin cellophane (0.00072 in.). D—intraperitoneally, 0.9 per cent sodium chloride solution 1.0 cc. per 100 grams.

those in which antidiuresis is produced by pituitrin in that the chief delay was not in the onset of water excretion but developed progressively after from 20 to 30 per cent of the ingested water had been excreted (fig. 7, *A* and *B*). It may be objected that the antidiuresis in these tests was the result of ineffective removal of other constituents of the urine by the modified dialysis. The sodium chloride concentration of the urine, however, measured in more than half the positive results, was never more than 1.2 per cent and was usually less than 0.4 per cent. Furthermore, the antidiuretic activity of these urines was abolished or greatly reduced by shaking with Lloyd's reagent. Among the 25 urines which gave these positive results were 5 from animals which had been completely pituitarec-

tomized by Dr. Curt P. Richter. Whatever the antidiuretic substance in those urines may have been it was not derived from the posterior lobe of the pituitary gland.

4. Differences with respect to antidiuretic activity between urines from hydrated and dehydrated rats are not apparent when the urine injections are made intraperitoneally rather than subcutaneously. Twenty specimens were collected from 16 groups of normal and 4 of hypophysectomized rats; 13 of the groups were hydrated, 7 dehydrated. Injections of 1 cc. per 100 grams were made into 51 groups of recipients. Eight of the specimens were dialyzed through 0.00072 inch cellophane for 3 hours, the sodium chloride concentrations being reduced to less than 0.4 per cent. The others were not dialyzed because it was found that the antidiuretic effect could usually be secured without preliminary concentration. Seventeen of the 20 specimens exhibited antidiuretic activity similar to that of pituitrin; the effect was quite as great with the urine of hydrated as of dehydrated animals when specimens representing an equal period of excretion were injected, and was not diminished by total pituitarectomy (fig. 7 *C*). The antidiuretic effect of these urines was abolished by filtration through collodion (2 expts.) or by shaking with activated charcoal (5 expts.). The injection of 0.9 per cent sodium chloride solution produced no antidiuretic effect in 14 trials (fig. 7 *D*). These results are similar to those of Arnold (29) whose injections of dog urine were all apparently made intraperitoneally.

5. The evidence summarized in the preceding sections, under headings 1 to 4, have made us doubt that the antidiuretic substance in dehydrated rat urine is secreted by the pituitary gland or is causally connected with the low urine volumes produced by dehydration. In sections 1 and 2, it has been shown that the antidiuretic substance differs from the antidiuretic fraction of pituitrin in its stability in rat urine and that rats are relatively unable to excrete the latter fraction in their urine. In sections 3 and 4 it has been shown that when the conditions of dialysis or the route of injection are altered an antidiuretic effect may be demonstrated in the urine of hydrated as well as dehydrated animals and that this effect is not due to a secretion by the posterior lobe of the pituitary gland since it is unaffected by total pituitarectomy.

Nevertheless the fact remains that if the technique of Gilman and Goodman is rigidly followed or if our own collodion adsorption method is used the urine of rats, as distinguished from that of the other animals we have examined, does contain some antidiuretic substance after dehydration which is absent when the animals are hydrated. Granting that this substance is not derived from the pituitary gland it is still possible that it is causally related to the oliguria. The small amounts which we have recovered however—5 to 10 per cent of that described by Gilman and Goodman—argue against its physiological importance here as they did in the

case of the other species unless it be assumed that the rat kidney is as ineffective in excreting antidiuretic substances as it is in excreting the antidiuretic fraction of pituitrin. And we find another point against its physiological importance in the lack of correlation which appears when its amount in rat urine is related to the urine volume of the animals as is done in the 3 typical experiments which are summarized in table 3.

REGULATION OF URINE VOLUME BY PITUITARECTOMIZED ANIMALS. In the experiments thus far described, by failing to isolate a substance which we could identify with the antidiuretic fraction of pituitrin from the various body fluids of animals which we have examined, we have failed to find affirmative support for the pituitary regulation of water diuresis. This line of evidence aside, the strongest argument favoring a pituitary control of urine

TABLE 3

Rat urine—Relation between volume and amount of antidiuretic substance

STATE OF HYDRATION	URINE VOLUME, CC./RAT	ANTIDIURETIC SUBSTANCE, MU PER RAT
Hydrated (drank 8 cc./rat)	2.1	0
24 hours dehydrated	2.4	2
48 hours dehydrated	1.3	7
72 hours dehydrated	1.3	10
24 hours dehydrated	2.0	0
48 hours dehydrated	2.0	6
72 hours dehydrated	1.3	6
96 hours dehydrated	1.0	0
48 hours dehydrated	2.2	15
72 hours dehydrated	1.7	20
96 hours dehydrated	1.6	8

volume lies in the observation that a chronic polyuria follows ablation of the posterior lobe by experiment or disease. There is however no compelling reason to identify the mechanism of this chronic polyuria with that of water diuresis; it is clear that some mechanism other than the pituitary gland is capable of affecting water reabsorption by the renal tubules since the urine volume in diabetes insipidus never equals that of the glomerular filtrate (6); and the argument would appear to lose its force if it could be shown that completely pituitarectomized animals respond to water administration and water deprivation quite as effectively after operation as before operation. We have made this demonstration on cats and rats and thereby somewhat extended the observations of Fee (30) and of Newton and Smirk (31) who showed that diuresis could be produced in hypophysectomized and decerebrate dogs and cats by the oral administration of water in the hours immediately following the operation.

Cats. The 24-hour urine volumes of a series of normal cats was measured in two-day periods during which the animals received 300 cc. of water per day by stomach tube ("diuretic"), had free access to water ("hydrated") and had no access to water ("dehydrated"). These measure-

TABLE 4

Effect on 24-hour urine volume of changes in water intake

A. Cats, before and after pituitarectomy

CAT NUMBER	PREOPERATIVE			POSTOPERATIVE		
	Diuretic	Hydrated	Dehydrated	Diuretic	Hydrated	Dehydrated
	Cc. of urine per twenty-four hours					
1	285 (360)	89	30	404 (400)	83	38
3	352 (300)	130	43	205 (325)	133†	42†
4	335 (260)		85*	234 (325)	133†	40†
5	327 (300)		83*	230 (320)	75†	40†
2	300 (300)	120	50			
424–24				300 (350)	74	29
427				359 (400)		
25					242	38
Averages...	320 (304)	113	51	289 (353)	123	38

All animals were offered 100 grams tinned salmon per day; several of them ate less well postoperatively.

Figures in brackets in columns 2 and 5 indicate average water intake per day.

* Average high because output maintained for first day of dehydration.

† Figure derived from one rather than two days' output.

B. Rats, normal and after pituitarectomy

	URINE VOLUME PER RAT PER DAY WHEN:		
	Hydrated	Dehydrated 24 hours	Dehydrated 48 hours
	cc.	cc.	cc.
Normal animals*...	7.9	3.4	2.2
Operated animals†...	10.8	5.9	3.1

* Figures derived from a large series of experiments performed during 1937; more recently, without obvious reason, the averages have been lower than this.

† The first figure is the average of 4 observations on 13 rats; 8 other animals, observed sooner after operation, gave a higher average than this. The other figures are the average of 6 observations on 21 animals.

ments were repeated in the same animals 4 to 9 days after total pituitarectomy had been performed by Dr. F. D. W. Lukens.[7] The results of 4

[7] At autopsy the pituitary fossa, in 7 of the 8 animals, was completely clean or contained small fragments of tissue consisting of nothing but necrotic fibrous tissue

complete and 4 incomplete experiments, appearing in table 4-A, disclose only minor differences in the response to water ingestion, apparently due to the fact that some of the animals ate less well postoperatively. The response to water withdrawal was entirely unaffected by operation and the animals which were in the best condition postoperatively withstood the withdrawal quite as well as normal cats. Similar reductions in urine volume in the case of cats with diabetes insipidus have been described by Fisher, Ingram and Ranson (7), and have been observed by ourselves in cats and dogs.

Rats. Water was administered by mouth in dosage of 5 cc. per 100 grams of body weight to 8 rats, completely pituitarectomized by Dr.

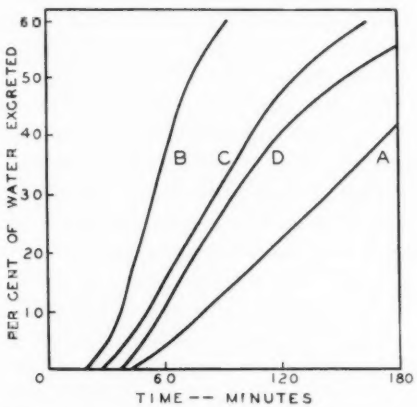


Fig. 8. Water diuresis in normal and pituitarectomized rats. Five cubic centimeters per 100 grams of water by mouth administered to: A—pituitarectomized rats, 3 weeks after operation; average of 8 experiments. B—normal rats; average of 163 experiments. C—pituitarectomized rats, 4 weeks after operation; average of 3 experiments. D—normal rats, half of water being given at 0, balance at 30 minutes; average of 5 experiments.

Curt P. Richter, during their third week after operation and again during the following week. The response to the first test was delayed and incomplete when compared with a different group of normal animals (fig. 8, *A* and *B*) but a week later it showed (fig. 8, *C*) only slight quantitative variation from the control. Such a variation can be produced in normal rats if the same amount of water be given in divided doses (fig. 8, *D*) and, occurring in the operated animals, it seems reasonable to ascribe it to an interference with absorption from the gastro-intestinal tract.

and leucocytes. In cat 1 small groups of pituicytes were observed by Dr. Balduin Lucké but their position and surroundings made their continued function unlikely.

The response to water deprivation was investigated in pituitarectomized animals after their recovery from the transient polyuria which follows the operation. As table 4-B indicates, urine volumes in the operated animals were somewhat greater at all stages of the experiment but their reduction on water withdrawal was comparable to that of the control animals. The specific gravity of specimens from the operated animals remained notably lower than that of the controls, the hydrated urines averaging 1015 instead of the normal 1030 and the dehydrated urines 1027 instead of the normal 1050; this distinction was not observed in 2 of 3 cats on which such measurements were made and we are ignorant of its mechanism.

The response of operated animals to water administration and withdrawal is thus so nearly identical with that of normal animals as to make it clear that some mechanism other than the posterior lobe of the pituitary gland is capable of effecting alterations in urine volume. It may be objected that a sufficient number of pituicytes survived in the median eminence to supply the operated animals with antidiuretic substance and the fact that the cats were excreting a normal volume of urine at the time of the experiments could be interpreted to support this view. To this objection, in so far as response to water ingestion is concerned, the experiments of Fee and of Newton and Smirk offer a better reply than do ours since theirs were performed upon animals with hypothalamus as well as pituitary gland removed; in so far as response to water withdrawal is concerned, the development of an oliguria in cats and dogs with diabetes insipidus would seem to answer the objection since the existence of the polyuria must be accepted as indicating the absence of antidiuretic hormone of any origin. Further, if the median eminence can serve as an adequate source of antidiuretic hormone, it is difficult to see why a posterior lobe pituitarectomy will produce chronic diabetes insipidus in rats (32).

SUMMARY

If the mechanism of water diuresis is to be attributed to variations in activity of the pituitary gland it seems most desirable to demonstrate an antidiuretic substance of pituitary origin in body fluids in concentrations which vary inversely with the degree of hydration of the animal. In this belief we have searched for such a substance in the blood, cerebrospinal fluid and urine of various animals following the administration and withdrawal of water. The search has been assisted by the development of a method for the separation of the antidiuretic fraction of pituitrin from the other constituents of cerebrospinal fluid and urine. The amount of antidiuretic substance which we have recovered has been assayed by the rat method of Burn and by a more sensitive method involving intravenous injection into diuretic rabbits.

In the case of rabbits' blood and rats' urine we demonstrated an antidiuretic effect which appeared to be peculiar to dehydrated animals and therefore to fulfill a requisite of the pituitary theory. In the one instance however recovery was not sufficiently constant to permit decision as to whether or not the substance originated in the pituitary gland; in the other, prolonged experimentation led us to conclude that the substance was probably not identical with the antidiuretic fraction of pituitrin, that the distinction between hydrated and dehydrated animals disappeared when the experimental conditions were modified, and that the substance was scarcely present in sufficient concentration to acquire physiological importance.

In the case of cerebrospinal fluid our experiments, though incomplete, support the definite conclusions which may be drawn from those upon the urine of cats, rabbits and dogs. They indicate that both fluids contain small amounts of an antidiuretic substance which does not originate in the posterior lobe of the pituitary gland since it is not abolished by removal of this lobe; this substance is not absent from the urine of hydrated or moderately diuretic animals and it cannot be considered responsible for the reduction in urine volume produced by dehydration, since its amount is not increased by this procedure. We are not aware of its origin.

These experiments have failed to disclose definitive support for the pituitary theory of water diuresis. Our regard for the theory is further lessened by the demonstration that pituitarectomized cats and rats continue to respond to water ingestion by water diuresis, and to water deprivation by oliguria, in a fashion quantitatively similar to that which they exhibited before operation.

To the considerable extent to which our conclusions have been reached by experiments on pituitarectomized animals we are indebted to the generous skill of Dr. F. D. W. Lukens of Philadelphia and Dr. Curt P. Richter of Baltimore. Miss Ethol Shiels of this department has done innumerable determinations of acidity and many analyses for sodium chloride and urea nitrogen. We are always in debt to Prof. A. N. Richards; in the present instance it is simple truth to say that the experiments would neither have been completed nor significant had it not been for his encouragement and suggestions.

REFERENCES

(1) Motzfeldt, K. J. Exper. Med. **25:** 153, 1917.
(2) Verney, E. B. Lancet **1:** 539, 1929.
(3) Klisiecki, A., M. Pickford, P. Rothschild and E. B. Verney. Proc. Roy. Soc. B. **112:** 521, 1933.
(4) Macleod, J. J. R. Physiology in modern medicine. 8th ed., C. V. Mosby Co., 1938, p. 965.

(5) Best, C. H. and N. B. Taylor. Physiological basis of medical practice. William Wood and Co., 1937, p. 614.
(6) Smith, H. W. Physiology of the kidney. Oxford Univ. Press, 1937, chapter XIX.
(7) Fisher, C., W. R. Ingram and S. W. Ranson. Diabetes insipidus. Edwards Bros., Inc. 1938.
(8) Marx, H. This Journal **96:** 356, 1931.
(9) Marx, H. Klin. Wchnschr. **5:** 92, 1926.
(10) Hart, P. D. and E. B. Verney. Clin. Science **1:** 367, 1934.
(11) Fee, A. R. J. Pharmacol. and Exper. Therap. **34:** 305, 1928.
(12) Buschke, F. Arch. f. Exper. Path. u. Pharmakol. **136:** 43, 1928.
(13) Theobald, G. W. and E. B. Verney. J. Physiol. **83:** 341, 1935.
(14) Pickford, M. J. Physiol. **95:** 226, 1939.
(15) Gilman, A. and L. S. Goodman. Science **84:** 24, 1936.
(16) Burn, J. H. Quart. J. Pharmacol. **4:** 517, 1931.
(17) Heller, H. and F. F. Urban. J. Physiol. **85:** 502, 1935.
(18) Marx, H. Klin. Wchnschr. **2:** 2384, 1930.
(19) Marx, H. and K. Schneider. Arch. f. Exper. Path. u. Pharmakol. **176:** 24, 1934.
(20) Melville, K. I. J. Exper. Med. **65:** 415, 1937.
(21) Anselmino, K. J. and F. Hoffmann. Klin. Wchnschr. **10:** 1438, 1931.
(22) Theobald, G. W. Clin. Science **1:** 225, 1934.
(23) Gilman, A. and N. E. Kidd. J. Pharmacol. and Exper. Therap. Proc. **63:** 10, 1938.
(24) Walker, A. M. Proc. Soc. Exper. Biol. and Med. **39:** 105, 1938.
(25) Ingram, W. R., L. Ladd and J. T. Benbow. This Journal Proc. **123:** 107, 1938.
(26) Gilman, A. and L. S. Goodman. J. Physiol. **90:** 113, 1937.
(27) Boylston, G. A. and A. C. Ivy. Proc. Soc. Exper. Biol. and Med. **38:** 644, 1938.
(28) Heller, H. J. Physiol. **89:** 81, 1937.
(29) Arnold, O. Arch. f. exper. Path. u. Pharmakol. **190:** 360, 1938.
(30) Fee, A. R. J. Physiol. **68:** 39, 1929.
(31) Newton, W. H. and F. H. Smirk. J. Physiol. **81:** 172, 1934.
(32) Richter, C. P. This Journal **110:** 439, 1934.

EFFECT OF THE SUBCUTANEOUS ADMINISTRATION OF WATER AND CERTAIN SALTS ON URINE VOLUME[1]

ARTHUR M. WALKER

From the Laboratory of Pharmacology, University of Pennsylvania, Philadelphia

Received for publication July 18, 1939

1. *Water.* It has proved rather more difficult to produce diuresis by the intravenous injection of water (1, 2) than would be anticipated if dilution of blood plasma electrolytes constitutes the mediate or immediate cause of the increased urine volume. The difficulty was overcome by Newton and Smirk (3) and their experiments finally disposed of the necessity for considering a gastro-intestinal hormone (4) in the mechanism of the diuresis. Our results confirm their experiments by a very simple technique and may have some advantages over theirs in that the subcutaneous route of administration obviates hemoglobinuria and permits injection of sufficiently large amounts of fluid so that the diuresis may be compared with that produced by oral administration of the same amount of water. The substitution of rats for rabbits provides, in our opinion, a much more reliable basis for the assay of diuretic effects.

Curve *A* (fig. 1) displays the average result of 9 experiments in which 2.5 cc. of distilled water per 100 grams of body weight were injected subcutaneously into groups of 4 animals and their urine excretion observed. The amounts of urine far exceed those produced by uninjected animals or by animals which have received similar injections of 0.9 per cent sodium chloride solution (curve *B*). The diuresis is less prompt and less marked than that which follows the oral administration of the same amounts of water (curve *C*) but this quantitative difference nearly disappears if the oral injection be divided into 2 doses (curve *D*); both diureses are similarly inhibited by morphine sulfate.

The subcutaneous administration of water to rats thus produces a diuresis closely analogous to that produced by its oral administration. Such difference as exists seems explicable on the basis of more rapid absorption from intestine than from subcutaneous tissue and we therefore conclude that the gastro-intestinal tract plays no specific rôle in the mechanism of water diuresis.

2. *Hypertonic solutions.* The intravenous injection of solutions which are hypertonic to blood plasma produce diuresis. We know of no record

[1] The expenses of this investigation were defrayed in large part from a grant from the Commonwealth Fund.

of the effect of the subcutaneous injection of such solutions upon urine volume and encountered an antidiuretic effect from sodium chloride as a source of error in another investigation. The observation was extended and the results of a series of 25 experiments appear in figure 2. When 1 cc. of 5 per cent sodium chloride solution per 100 grams of body weight was injected subcutaneously into a group of 4 rats immediately after they re-

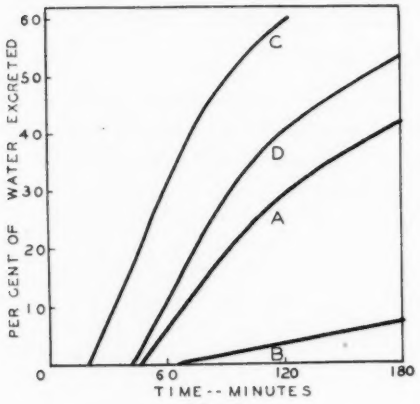


Fig. 1

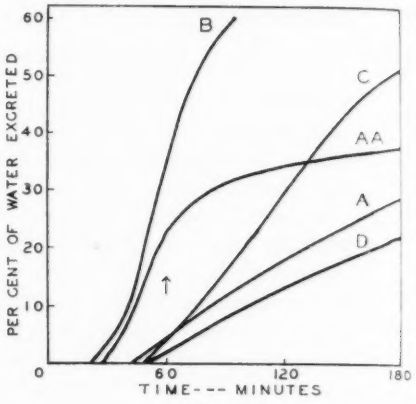


Fig. 2

Fig. 1. Effect of administration to rats, 2.5 cc. per 100 grams of body weight, of: *A*—water subcutaneously; average of 9 experiments. *B*—0.9 per cent NaCl solution subcutaneously; average of 15 experiments. *C*—water by mouth; average of 9 experiments. *D*—water by mouth, one-half at *O*, balance at 30 minutes; average of 5 experiments.

Fig. 2. Effect of subcutaneous injection of hypertonic solutions on water diuresis. In all experiments except those of curve *D* each group of rats received water by mouth, 5 cc. per 100 grams of body weight, at 0 minute. The normal response is the diuresis exampled by curve *B*. Additional injections were made as follows: *A*—1 cc. per 100 grams 5.0 per cent sodium chloride solution subcutaneously at 0 minute. Average of 8 experiments. *AA*—Same as curve *A* at point indicated by arrow. Average of 2 experiments. *B*—1 cc. per 100 grams 6.2 per cent calcium chloride or 6.4 per cent potassium chloride or 10 per cent urea solutions subcutaneously at 0 minute. Average of 7 experiments. *C*—1 cc. per 100 grams 21.5 per cent sodium sulphate or 32 per cent glucose solutions subcutaneously at 0 minute. Average of 8 experiments. *D*—5 cc. per 100 grams 1.0 per cent sodium chloride solution by mouth at 0 minute. No subcutaneous injections. Average of 9 experiments.

ceived 5 cc. of water per 100 grams by mouth (curve *A*), or shortly after the water diuresis commenced (curve *AA*), the development of the diuresis was delayed or arrested in comparison with that of control animals receiving a similar injection of 0.9 per cent sodium chloride solution (curve *B*). Solutions of sodium sulphate or glucose which were isosmotic with the hypertonic sodium chloride produced less marked antidiuresis (curve *C*), and

similar solutions of calcium chloride, potassium chloride and urea were without antidiuretic effect, the excretion curve being superimposable on that of the control groups (curve *B*). We did not observe any withdrawal of fluid from blood to tissue at the site of injection and the animals gave no constant evidence of pain.

The mechanism of this antidiuretic effect, in so far as the sodium chloride solutions are concerned, presumably lies in their preventing the plasma electrolyte dilution which the administration of water alone produces; and is thus analogous to the marked inferiority of 0.9 per cent sodium chloride solution to water as a diuretic when the two solutions are given in equivalent amounts by mouth, an effect which has been repeatedly recognized (5, 6) and is easily demonstrable on rats (curve *D* vs. *B*). The effect is probably not due to an increased production of antidiuretic hormone by the pituitary gland (7) for it continued to occur in 2 experiments upon totally pituitarectomized animals.

The effect of the other solutions are recorded without comment except to say that the difference between potassium and sodium chloride has been recognized when the two solutions have been given by mouth (5).

SUMMARY

The effects of the subcutaneous injection of water into rats confirms existing evidence as to the unimportance of the gastro-intestinal tract in the mechanism of water diuresis.

The subcutaneous injection of certain hypertonic solutions in rats has been found to delay or arrest the development of a water diuresis.

REFERENCES

(1) Fee, A. R. J. Pharmacol. and Exper. Therap. **34:** 305, 1928.
(2) Verney, E. B. Lancet **1:** 539, 1929.
(3) Newton, W. H. and F. H. Smirk. J. Physiol. **78:** 451, 1933.
(4) Cow, D. J. Physiol. **48:** 1, 1914.
(5) Adolph, E. F. This Journal **65:** 419, 1923.
(6) Cushny, A. R. The secretion of urine, Longmans, Green and Co., 1926, p. 168.
(7) Gilman, A. and L. S. Goodman. Science **84:** 24, 1936.

THE EXCRETION OF ANTIDIURETIC SUBSTANCE AND ITS RELATION TO THE HYPOTHALAMICO-HYPOPHYSEAL SYSTEM IN CATS

W. R. INGRAM, LAURA LADD AND J. T. BENBOW

From the Department of Anatomy, State University of Iowa

Received for publication June 26, 1939

The functions of the nervous pathway between the supraoptic nucleus of the hypothalamus and the pars nervosa of the pituitary gland offer some interesting problems. Interruption of this bundle of nerve fibers results in atrophy of the pars nervosa and changes in the cellular constituents of the latter. Sufficiently extensive alterations are accompanied by the onset of diabetes insipidus, which is generally held to be due to lack of an antidiuretic hormone normally produced by the neurohypophysis (Fisher, Ingram and Ranson, 1938). Is this innervation of the posterior lobe a trophic one or does it offer a means of regulating the activities of the gland and varying the output of its active principles? Variations in the amount of antidiuretic substance acting upon the kidney have been suggested by various workers. Quantitative determinations of posterior pituitary principles in the body fluids are difficult and there is evidence that the antidiuretic hormone is inactivated by blood and tissues (Heller and Urban 1935). However, Theobald and Verney (1935), Haterius (1939), Pickford (1939) and others have advanced indirect evidence for humoral and neural influences causing the posterior lobe to increase its putput of antidiuretic substance. Chang et al. (1938) have offered evidence that pressor substance is released by the hypophysis in response to stimulation of afferent vagus fibers. The most direct evidence, however, so far as antidiuretic substance is concerned, is that of Gilman and Goodman (1937). Following the observation of Heller and Urban (1935) that some part of injected antidiuretic substance may be excreted in the urine, in which its concentration can be estimated by bioassay, Gilman and Goodman found that dehydrated rats excreted large quantities of this material, while normal animals failed to excrete any. The urine from dehydrated hypophysectomized rats did not contain antidiuretic substance. These observations were not confirmed by Walker (1938), but Boylston and Ivy (1938) corroborated them in regard to the effect of dehydration of normal rats. Ingram, Ladd and Benbow (1938) reported similar findings for cats. The present communication presents in some detail experiments relating to

the excretion of antidiuretic substance by normal cats and cats with diabetes insipidus under various conditions.

METHODS. The experimental cats used were adults of average size and weight. Diabetes insipidus was produced by interrupting the supra-optico-hypophyseal tract, the electrolytic lesions being placed with the aid of the stereotaxic instrument as described by Fisher, Ingram and Ranson; the general results have been extensively discussed by these authors. All the cats were kept on a constant diet of fresh meat and milk. Careful records of urine output and food and fluid intake were kept. The diabetes insipidus cats were dehydrated by simply withholding all fluid and all but a little solid food. In normal cats, however, a state of dehydration was not always easily induced by withholding fluid; although they may be dehydrated in this way, some cats remain in very good condition after many days without water and subsisting on small quantities of meat cooked dry. Quicker results were obtained by purging the cat with $MgSO_4$ given by stomach tube, followed on the next day by intravenous injection of 2.5 to 4.0 grams of NaCl in 10 per cent solution. Under such treatment combined with fasting and fluid deprivation the erythrocyte count increased by 20 to 50 per cent. In some experiments 10 to 20 cc. of 50 per cent sucrose was injected, but with less positive results. These procedures were not without danger, but were supported well by most cats. They were not applied to diabetes insipidus cats because of the risk to such fairly valuable animals and because such cats become severely dehydrated soon after water deprivation. Twenty-four hour urine specimens were collected in glass receptacles containing small quantities of acetic acid. The urine was collected from time to time during the day and stored in the refrigerator.

For the determination of antidiuretic substance in the urine the method of Burn as described by Gilman and Goodman was used. The 24 hour urine specimens were dialyzed, using cellophane tubular membranes, for $6\frac{1}{2}$ to 7 hours. This time was found to be most suitable for cat urine, which, in our experience, may frequently contain material which is decidedly toxic to rats and which may still be present after 3 hours' dialysis. A large number of experiments were spoiled by this factor. This toxic material has a marked depressor effect upon the blood pressure of anesthetized cats, and apparently depressses kidney activity in rats. Needless to say, all experiments were discarded in which there was indication of traces of this substance in the urine. Many urine samples were examined by testing their influence upon cats' blood pressure and others were checked by their effects upon rats. Dialysis for $6\frac{1}{2}$ to 7 hours or even longer was found to be without appreciable influence upon the antidiuretic activity of solutions containing small quantities of Pitressin. The same was true when Pitressin was added to normal cat urine and dialyzed. After dialysis the acidity of the urine samples was checked and if deficient, acetic acid

was added. The samples were then concentrated in vacuo at 45°C. to volumes ranging from 20 to 85 cc. Experience showed the larger volume to be quite suitable for testing.

For the actual determination of antidiuretic content groups of 4 male rats were used. As described elsewhere (Heller and Urban, 1935) the principle involves the effect of small doses of urine injected subcutaneously upon a diuresis produced by the oral administration of 5 cc. water per 100 grams body weight. The procedure was as follows. Several hours before the experiment the rats were given a priming dose of water (2.5 cc. per 100 grams of rat). At the time of the experiment the test dose of water was given, the urine injected subcutaneously (1 cc. per 100 grams weight in the early experiments, 2.5 cc. per rat in the later) and the urine output of the group measured every 15 minutes until a volume of urine equivalent to one half the test dose of water had been excreted. The total interval has been called the inhibition time (Heller and Urban, 1935), and furnishes an indication of the presence or absence of antidiuretic substance. Comparison of the inhibition times following urine administration with those subsequent to injection of known quantities of Pitressin gives an indication of quantity of antidiuretic substance present. The experiments were carried out at room temperatures of approximately 25°C.

In the early experiments the groups of rats were chosen at random and the determinations standardized and controlled on the basis of average inhibition times. The inhibition times of these varying groups were determined after subcutaneous injection of 5, 10 and 15 milliunit doses of Pitressin[1] per rat, after injection of distilled water and after administration of normal urine as well as urine from dehydrated cats. In later experiments constant groups were used, each group being standardized for its particular response to posterior lobe extracts, plain water and normal urine from control and diabetes insipidus cats. The latter groups presumably gave a more accurate estimate of quantity of antidiuretic substance in individual assays. The method of averages was also applied in this second series.

Results. *First series.* The inhibition times of the various types of experiments were grouped, averages taken and standard errors calculated. The validity of the averages was checked by Fisher's "test of t" (Fisher, 1936). The results may be summarized as shown in table 1.

It will be noted that the inhibition times following injection of normal urine are somewhat greater than in the control experiments. Statistical check of the difference between (1) and (5) according to Fisher's method suggests that it is possibly barely significant ($P = > 0.05$). The difference between (1) and (6) is statistically significant. There is no signifi-

[1]Our thanks are due Dr. Oliver Kamm, of Parke Davis and Co., for a generous supply of Pitressin.

cant difference, however, between (5) and (6), so that while cat urine may, according to this series of experiments, contain minute quantities of some substance capable of prolonging the inhibition time, there is no difference between that of normal animals and cats with diabetes insipidus. Urine from diabetes insipidus cats in marked dehydration caused no increase in inhibition time (7). Dehydration of these animals was severe enough to cause death in one case.

Normal cats, when dehydrated, however, excreted urine which prolonged the inhibition time considerably. Statistically there is no significant difference between (2) and (8), so there is justification for stating that urine from dehydrated normal cats, after dialysis and concentration may contain antidiuretic substance equivalent to approximately 5 milliunits of Pitressin per 2.5 cc., on the average. Construction of a composite standard curve in which inhibition times are plotted against Pitressin dose per rat furnishes a basis for estimation of the antidiuretic activity of urine from

TABLE 1

MATERIAL INJECTED	NUMBER OF EXPERIMENTS	INHIBITION TIME
		minutes
(1) Water	17	97 ±3
(2) 5 mU Pitressin per rat	18	155 ±5.5
(3) 10 mU Pitressin per rat	8	166 ±8.4
(4) 15 mU Pitressin per rat	3	215
(5) Normal cat urine	8	111 ±7.3
(6) Diabetes insipidus cat urine	10 (6 cats)	115 ±6.2
(7) Urine from dehydrated d.i. cats	5 (3 cats)	112 ±2.3
(8) Urine from dehydrated normal cats	18 (6 cats)	146 ±7.6

individual cats under various conditions, the experimental inhibition times being super-imposed upon the standard curve and the total day's output of antidiuretic substance calculated. The average output in this series of dehydrated cats was approximately 0.82 milliunit per cubic centimeter of urine, varying from 0.4 to 1.8. The smallest total daily output was 20.0 mU, the largest 420 mU. Sometimes in running a dehydration experiment a sample of urine was obtained which apparently contained no antidiuretic substance even though the same cat on previous occasions and thereafter did excrete antidiuretic substance. The reason for these occasional failures cannot be stated with certainty. Imperfection in technique may account for them—possibly the specimens were not kept sufficiently acid to preserve the antidiuretic substance. These negative samples have been included in computing the average given under (8) in table 1.

It should be mentioned that the dehydration experiments were controlled not only by comparing inhibition times of urine from dehydrated cats with

those of urine from other, normal cats, but also in nearly all cases by testing urine from the same animals when the fluid balance was normal. It should also be noted that normal cats subjected to simple fluid deprivation may excrete antidiuretic substance; the more drastic methods of dehydration were used in most cases, however.

Second series. In this series groups of the same four rats were used, the groups being segregated and individually standardized. An entirely different batch of rats from those in series one was used. No dehydrated diabetes insipidus cats were included in these experiments, the chief consideration being confirmation of the findings in regard to normal animals. The results are summarized as shown in table 2.

These averages are also statistically significant. It is interesting that in this series as contrasted with series one, there was no difference between the inhibition time of the controls (1) and that produced by normal urine (4). The inhibition times were in general shorter in this series. Nevertheless, the average inhibition time of dehydrated cats' urine was again

TABLE 2

MATERIAL INJECTED	NUMBER OF EXPERIMENTS	INHIBITION TIME
		minutes
(1) Water	24	91 ±1.7
(2) 5 mU Pitressin per rat	18	126 ±3.8
(3) 10 mU Pitressin per rat	17	154 ±3.6
(4) Normal cat urine	13	94 ±2.1
(5) Urine from dehydrated normal cats	8 (6 cats)	126 ±5.3

equivalent to that produced by injection of 5 milliunits of Pitressin. Here again, as in series one, inhibition times of individual cats' urine obtained under normal conditions were compared with those produced by urine from the same cats under dehydration conditions, and as checked by Fisher's formulae the differences were significant. In several instances the results were re-checked by assaying the urine after the cat had been restored to normal fluid intake. A few experiments gave negative results—in some of these the urine was found to be quite alkaline and hence unsuitable for preservation of antidiuretic substance, in others negative results after a single intravenous injection of salt or sucrose were later followed by positive effects. The average urine content of antidiuretic substance was approximately 1.5 milliunits per cubic centimeter, varying from 0.5 to 3.6. These values were computed from standard curves for each test group of rats.

Curves illustrating a typical experiment are shown in figure 1.

In a few cases assays were made of urine from animals receiving posterior

lobe injections. Two cats with polyurias of 400–500 cc. per day were treated with large doses of Pitressin (total of 0.9 cc. or 18 units in 3 injections) so that their urine volumes were reduced to approximately normal levels (112 and 170 cc.). In each case antidiuretic substance was excreted so that the inhibition times were greatly prolonged over those obtained

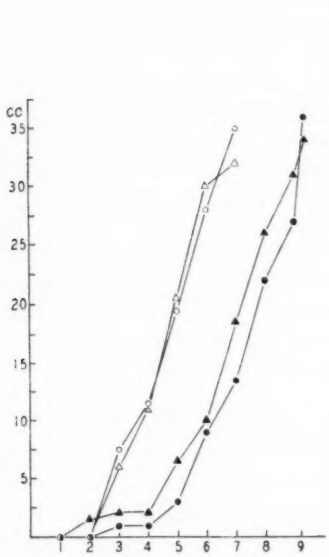


Fig. 1

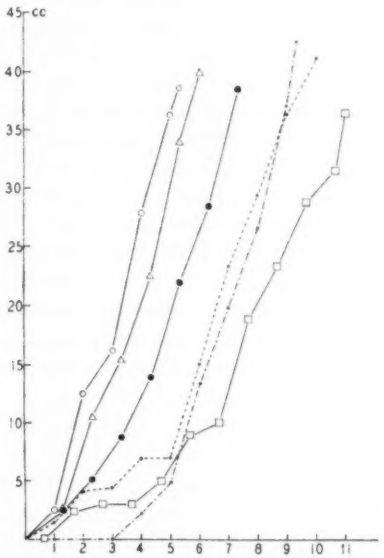


Fig. 2

Fig. 1. Effect of dehydration of a normal cat upon excretion of antidiuretic substance. Along the ordinate, cubic centimeters of urine excreted by the test group of 4 rats. Along abscissa, time in 15 minute intervals. Circles, control water diuresis of test group II. Solid circles, effect of 5 milliunits of Pitressin per rat. Triangles, effect of injection of normal urine from cat N12. Solid triangles, effect of injection of urine from cat N12 during dehydration.

Fig. 2. The excretion of antidiuretic substance after administration of Pitressin to cats with diabetes insipidus. Circles, control water diuresis of test group I a. Solid circles, effect of 5 milliunits of Pitressin per rat. Squares, effect of 10 milliunits of Pitressin per rat. Triangles, effect of injection of normal cat urine. Interrupted lines, effect of injection of urine from cats with diabetes insipidus under treatment with Pitressin.

from ordinary urine from the same animals. In one case the antidiuretic activity was equivalent to 3.1 milliunits per cubic centimeter, in the other it was equivalent to 3.8 milliunits per cubic centimeter. The total excreted was far below the quantity injected. Figure 2 illustrates graphically these experiments.

Normal cats receiving Pitressin also excrete antidiuretic substance in

the urine. Two animals were given 0.9 cc. Pitressin daily for 4 days. The urine from the last day was tested. In one case the inhibition time was 124 minutes, equivalent according to the rat group used to 2.4 milliunits of Pitressin per cubic centimeter. Considerably more antidiuretic substance was present in the other urine specimen—the experiment was discontinued after 170 minutes with the excretion incomplete.

Discussion. The data presented indicate that the normal cat subjected to dehydration excretes appreciable quantities of antidiuretic substance in its urine. Certain quantitative differences between the above observations and those of other investigators remain unexplained. The amounts of antidiuretic substance excreted by dehydrated cats were relatively much less than those excreted by rats under somewhat similar conditions (cf. Gilman and Goodman). Furthermore, it is not entirely clear as to why a relatively small part of a subcutaneous dose of commercial Pitressin is excreted. The large proportion of the injected material which is retained may have been destroyed by the tissues, as suggested by Heller. Gilman and Goodman reported that antidiuretic substance appeared in rat urine only after more than 1 unit per 100 grams of rat had been injected. Our cats received considerably less than this (approximately 0.4–0.6 unit per 100 grams). A species difference in rate of destruction or excretion may explain this discrepancy.

In spite of these differences our observations support the contention of Gilman and Goodman that antidiuretic hormone may be produced in excess of normal when there is need for conservation of body water. Cats with diabetes insipidus due to interruption of the supraoptico-hypophyseal tract, without direct injury to the posterior lobe, fail to excrete such additional substance when dehydrated. Since this indicates a breakdown of the normal mechanism in the absence of the innervation of the pars nervosa, it seems logical to postulate that the supraoptico-hypophyseal nerve connections form the efferent limb of a "reflex" mechanism concerned in the hormonal regulation of water excretion by the kidney. That the nervous connections are the important factor is shown by the fact that the posterior lobe is still present and is unable of itself to respond to the stimulus of the need for water retention. It should not be forgotten, however, that the denervated neurohypophysis is practically totally lacking in the usual posterior lobe principles, as shown by extraction (Fisher and Ingram, 1936) and that it has undergone certain histological changes. The idea of this "reflex" mechanism is rather an attractive one, nevertheless, and in addition to evidence previously mentioned, further support is added by the cytological observations of Gersh (1939), who found definite changes in the specific parenchymatous cells of the neurohypophysis of the rat in response to lack of water. The mode and point of action of the stimulus involved on the "afferent" side, presumably in itself a change in the water

content of the body fluids, can only be surmised. Does this change make itself felt peripherally, at nerve endings, and do afferent impulses reach the supraoptic nucleus by ascending pathways through the brain stem (cf. Huang, 1938)? Perhaps the cells of the supraoptic nucleus may themselves be directly activated by alteration in the fluid constituents of the body.

SUMMARY

Normal cats subjected to conditions producing dehydration excrete appreciable quantities of antidiuretic substance in the urine. The presence of this substance may be detected by bioassay procedures with rats as test animals, according to the method of Burn as used by Heller and by Gilman and Goodman.

Cats with diabetes insipidus due to interruption of the supraoptico-hypophyseal tract without direct injury to the hypophysis, do not excrete antidiuretic substance when dehydrated.

When commercial post-pituitary preparations are injected in normal cats and cats with diabetes insipidus, antidiuretic substance appears in the urine in quantities which are relatively small as compared with the amount administered.

The results are interpreted as offering support for the suggestion of Gilman and Goodman that the neurohypophysis may respond to the need for water conservation by secreting increased amounts of antidiuretic hormone, and indicate that the supraoptico-hypophyseal tract is necessary for this response.

REFERENCES

(1) Boylston, G. A. and A. C. Ivy. Proc. Soc. Exper. Biol. and Med. **38:** 644, 1938.
(2) Chang, H.-C., K.-F. Chia, C.-H. Hsu and R. K. S. Lim. Chinese J. Physiol. **13:** 13, 1938.
(3) Chang, H.-C., R. K. S. Lim and Y.-M. Lü. Chinese J. Physiol. **13:** 33, 1938.
(4) Fisher, C. and W. R. Ingram. Endocrinology **20:** 762, 1936.
(5) Fisher, C., W. R. Ingram and S. W. Ranson. Diabetes insipidus and the neurohormonal control of water balance. Edwards Bros., Inc. Ann Arbor, Mich. 1938.
(6) Fisher, R. A. Statistical methods for research workers. Oliver and Boyd, Edinburgh, 1936 (6th ed.).
(7) Gersh, I. Am. J. Anat. **64:** 407, 1939.
(8) Gilman, A. and L. S. Goodman. J. Physiol. **90:** 113, 1937.
(9) Haterius, H. O. Proc. Am. Physiol. Soc., p. 112, 1939.
(10) Heller, H. and F. F. Urban. J. Physiol. **85:** 502, 1935.
(11) Heller, H. J. Physiol. **89:** 81, 1937.
(12) Huang, J. J. Chinese J. Physiol. **13:** 367, 1938.
(13) Ingram, W. R., L. Ladd and J. T. Benbow. This Journal **123:** 107, 1938.
(14) Pickford, M. J. Physiol. **95:** 226, 1939.
(15) Theobald, G. W. and E. B. Verney. J. Physiol. **83:** 341, 1935.
(16) Walker, A. M. This Journal **123:** 210, 1938.

THE RÔLE OF THE PITUITARY IN THE CALORIGENIC ACTION OF VITAMIN D[1]

A. BARTOLI,[2] J. FELDMAN AND C. I. REED[3]

From the Department of Physiology, University of Illinois, College of Medicine

Received for publication June 14, 1939

It has been noted repeatedly that deficiency of vitamin D will definitely decrease the metabolic rate (Seel, 1929; Thoenes, 1930; Landelius and Ljugkvist, 1934; Nitschke, 1933); and that subsequent administration of vitamin D will again increase the rate (Quadri, 1933; Nitschke and Schneider, 1932).

Also it has been shown that large but subtoxic doses of vitamin D will increase the metabolic rate of normal dogs (Reed, Thacker, Dillman and Welch, 1933; Gormaghtigh and Handovsky, 1935) and albino rats (Reed, 1934). However, Sherwood, Toth and Carr (1934) failed to produce any calorigenic action from feeding cod liver oil to rats. Lands and Stoland (1935) found histological changes in the thyroid glands of cats after toxic doses of vitamin D but were doubtful of their significance.

Steck, Miller and Reed (1934) demonstrated that this reaction was not due to stimulation of the parathyroids by the vitamin since injection of large doses of parathyroid extract did not increase the metabolic rate. Deutsch, Reed and Struck (1936) showed that the calorigenic action of vitamin D is dependent upon the integrity of the thyroids. Also, it has been shown that large amounts of the vitamin are capable of increasing the oxygen consumption of isolated peripheral tissues in a manner comparable to that observed after thyroxin administration (Gelfan, 1935; Presnall, 1937).

In some preliminary experiments four dogs were hypophysectomized by the oral route. After establishing a basal level of metabolism for each dog, vitamin D in the form of Ertron was administered orally in amounts of 10,000 to 15,000 units per kilogram of body weight per day. After 10 to 21 days 3 of the dogs showed mild calorigenic reactions but the other

[1] A part of the expenses of this investigation was borne by grants from the Graduate School Research Fund and from the Nutrition Research Laboratories.

[2] Submitted in partial fulfillment of requirements for the degree of Master of Science, 1939.

[3] Valuable technical assistance was rendered by L. L. Fiorito, L. G. McKeever, H. C. Struck, B. O. Barnes and E. B. Plattner, for which grateful acknowledgment is given.

showed no change. At autopsy small amounts of pituitary tissue were found in each of these 3 animals, while none was found in the fourth. While the correlation was suggestive it was felt that this still was not conclusive.

In further experiments the operation was done by the temporal route. Post-operative training for metabolic readings required 3 to 5 weeks. A closed circuit outfit was adapted as previously described (Deutsch, Reed

TABLE 1

Dog B, daily average metabolic rate

	CAL./M.²/HR.	VITAMIN D UNITS/KILO/DAY	BLOOD	
			Ca. Mgm. per cent	Inorg. P mgm. per cent
Control	39.275	0	10.2	3.7
1 second treatment period	36.910	39,000	15.7	3.7
1 second rest period	30.070	0	17.3	3.4
			13.3	3.4
			11.2	3.5
			10.9	3.7
			10.9	3.7
			9.8	3.6
			9.6	3.6
			9.1	3.8
			9.6	4.6
			9.8	4.0
2 second treatment period	22.436	30,000	9.7	3.8
			10.2	4.3
			13.8	3.9
3 second treatment period*	21.875	42,000	17.3	4.1
			16.0	4.6
			14.2	3.8
			15.6	3.8
2 second rest period	21.885	0	14.0	3.9
			13.1	3.6
			12.5	3.7

* The differentiation between 2 and 3 second treatment periods is solely one of dosage as there was no intervening rest period.

and Struck, 1936). The surface area formula of Cowgill and Drabkin (1927) was used.

Protocol. Dog B, male, 20 pounds. Hypophysectomy, August 19, 1938; training instituted after 3 days. After control period, administration of vitamin D was instituted September 12. This consisted of 50,000 unit capsules of Ertron in doses averaging 39,000 units per kilo per day through October 11, at which time treatment was discontinued; weight, 16 pounds. Weight recovery by December 15. December 27 to January 10, 1939, 30,000 units per kilo per day. Some weight gain. Janu-

ary 11 to February 8, 42,000 units; weight 16 pounds. Sacrificed March 23, weight 20 pounds.

Autopsy. Gross appearance of organs; spleen enlarged; both subcutaneous and abdominal fat plentiful; skeletal muscle thin and glistening. Histological: thyroid, aorta normal; spleen, marked congestion; liver, hepatic cells large, cytoplasm coarsely granular, nuclei pale, sinusoids largely obliterated; kidney, glomeruli normal, slight tubular degeneration, most marked in collecting tubule; skeletal muscle, interstitial tissue decreased, local areas of fiber degeneration.

Pituitary stalk, small area of degenerated pituitary cells surrounded by fibrous tissue.

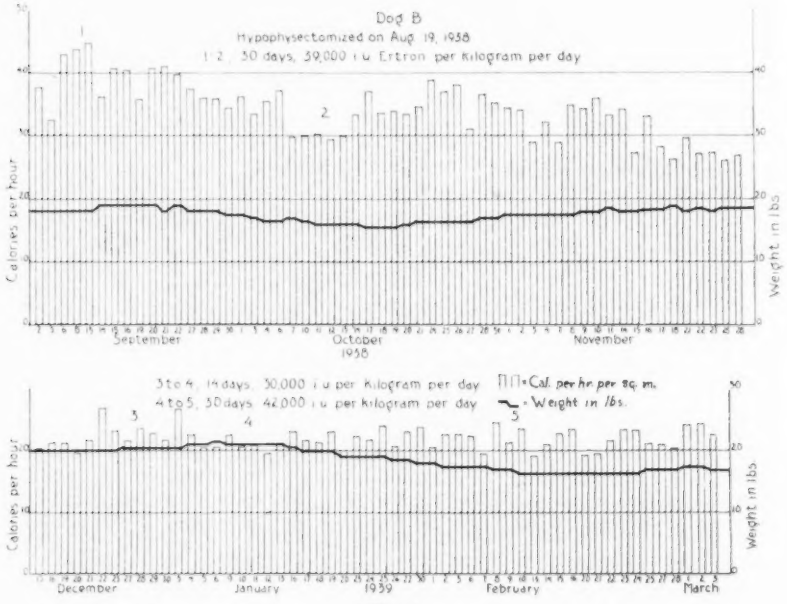


Fig. 1

The protocols of the remaining four dogs conform closely to dog B, hence they are omitted. All were carried for shorter periods after the operation—48, 42, 62 and 60 days respectively. In 2 of the 5 animals the pituitary had been completely removed; in the other three, while the cells were identifiable as of the anterior lobe, they were so completely degenerated as to be non-functional.

Effects on metabolism. Whereas in previous investigations doses of vitamin D much smaller than those used in this project invariably produced a large increase in metabolism in normal animals, it will be seen in table 1 and figure 1 that no such increase occurred in hypophysectomized dogs. On the contrary the general trend was downward so that the general

level was only about half that during the initial control period. The concentration of calcium in the blood was altered in about the same manner as in normal dogs while the inorganic phosphorus was not altered in any consistent manner. However, the reduction of hypercalcemia was much less rapid than occurs in normal dogs similarly treated, in our experience.

This supports our belief that the effects of vitamin D on mineral metabolism are largely independent of its calorigenic action, since the latter was completely absent in hypophysectomized dogs while the former was not greatly affected.

Another important observation is that the toxic action of vitamin D is greatly modified in hypophysectomized dogs. The weight loss in the latter seems to be largely due to muscular degeneration while in normal animals it is partly due to exhaustion of fat depots. Also the usual syndrome of toxicity as observed in intact dogs was absent in hypophysectomized dogs except for anorexia and weight loss.

This particular preparation of vitamin D is known to be less toxic than viosterol in that the threshold for normal dogs appears to be about 20,000 units per kilo per day for viosterol and 25,000 units for Ertron. However, the dosages used were sufficiently large to offset this difference so the absence of toxicity must be ascribed to the absence of the pituitary.

These experiments do not prove that vitamin D in optimal amounts influences pituitary function but only that the latter is necessary for the calorigenic response to large doses.

SUMMARY

1. Hypophysectomized dogs do not show a calorigenic response to vitamin D in doses sufficiently large as to produce a marked response in normal dogs.

2. The calorigenic response is largely independent of the effect of vitamin D on mineral metabolism.

3. Hypophysectomized dogs are less readily intoxicated by vitamin D than are intact dogs similarly treated.

4. Both the pituitary and the thyroid are necessary for the calorigenic response to vitamin D.

REFERENCES

COWGILL, G. R. AND D. L. DRABKIN. This Journal **81:** 36, 1937.

DEUTSCH, H., C. I. REED AND H. C. STRUCK. This Journal **117:** 1, 1936.

GELFAN, S. This Journal **113:** 464, 1935.

GOORMAGHTIGH, N. AND H. HANDOVSKY. Compt. rend. Soc. Biol. **118:** 1616, 1935.

LANDS, A. M. AND O. O. STOLAND. Endocrinology **19:** 701, 1935.

LANDELIUS, E. AND G. LJUNGKVIST. Skand. Arch. f. Physiol. **68:** 252, 1934.

NITSCHKE, A. Klin. Wchnschr. **12:** 1793, 1933; Ztschr. f. d. ges. exper. Med. **82:** 236, 1932.

NITSCHKE, A. AND M. SCHNEIDER. Ztschr. f. Kinderh. **52:** 671, 1932.
PRESNALL, A. K. J. Biol. Chem. **121:** 5, 1937.
QUADRI, S. La Clin. Ped., Sept., 679, 1933.
REED, C. I. Proc. Soc. Exper. Biol. and Med. **32:** 274, 1934.
REED, C. I., E. A. THACKER, L. M. DILLMAN AND J. W. WELCH. J. Nutrition **6:** 355, 1933.
SEEL, H. Arch. f. exper. Path. u. Pharmakol. **140:** 194, 1929.
SHERWOOD, T. C., L. A. TOTH AND K. CARR. Endocrinology **18:** 254, 1934.
STECK, I. E., D. S. MILLER AND C. I. REED. This Journal **110:** 1, 1934.
THÖENES, T. Jahrb. f. Kinderh. **126:** 348, 1930; **129:** 171, 1930.

STUDIES IN IODINE METABOLISM BY THE USE OF A NEW RADIOACTIVE ISOTOPE OF IODINE[1]

JOSEPH G. HAMILTON AND MAYO H. SOLEY

From the Divisions of Medicine and Pharmacology of the University of California Medical School, the Radiation Laboratory of the University of California, and the Thyroid Clinic of the University of California Hospital, San Francisco and Berkeley

Received for publication June 28, 1939

The recent discovery of three new radioactive isotopes of iodine by Livingood and Seaborg (1) has provided almost unlimited opportunities for the study of the metabolism of this element and its compounds in living organisms. Prior to this time the only radio-iodine[2] available for biological investigation was limited in its usefulness since its half-life[3] was only 25 minutes (2, 3, 4). This has confined studies making use of this material to a period of time of less than two hours. For the study of iodine metabolism, the most important of these newly discovered radio-iodines is that with an atomic weight of 131. This radio-element has a half-life of 8 days which permits it to be used for a period of several weeks. It should be kept in mind that any radioactive isotope of a given stable element differs from the element only in its property of being radioactive. So long as the concentration of the radio-element is not so great as to produce changes by the direct physical effects of its radiation, the isotope remains chemically and physiologically identical to the naturally occurring element. The radioactivity "tags" the atoms and, since the quantity of radioactive material used in metabolic studies is extremely minute in comparison to the intensities required to produce biological changes, it may be assumed that the course of radioactive isotopes in the body gives an index of the metabolism of the inactive isotopes.

The object of this investigation was to establish the rates of excretion of iodine in normal human subjects and in a small group of patients suffering from several different types of thyroid diseases; and, in addition, to determine the uptake of iodine in the thyroid glands of the patients who underwent thyroidectomy.

[1] Aided by grants from the Rockefeller Foundation of New York City, and the Christine Breon Fund for Medical Research. Acknowledgement is herewith made for services rendered by the Works Progress Administration, Project no. 10,695; and the National Youth Administration.

[2] The prefix "radio-" indicates that the element so described is radioactive.

[3] The "half-life" is the length of time required for half of a given number of activated atoms of a radio-element to disintegrate.

Preparation of the radio-iodine. The radio-iodine was prepared by the deuteron bombardment of from 1 to 2 grams of metallic tellurium. In addition to the 8-day radio-iodine, there are four other radioactive iodine isotopes produced by the action of the deuterons upon the tellurium. However, these latter were of no great importance in the studies undertaken since two of them have relatively short half-lives; and the other two, whose half-lives are 4 and 13 days respectively, are formed in small amounts as compared to the 8-day isotope.

Following the deuteron bombardment in the cyclotron, the tellurium was removed from the target and was placed in a distilling flask equipped with a long delivery tube. Thirty cubic centimeters of 6N nitric acid were then added to the flask and 10 cc. of 0.008N sodium iodide solution were poured into the mixture. The flask was then heated and the free iodine distilled over into a receiver containing 100 cc. of carbon tetrachloride. The nitric acid quickly dissolved the tellurium and released the radio-iodine, which was carried over as iodine vapor with added inert iodine into the carbon tetrachloride. After the tellurium had been dissolved completely and all of the iodine distilled over, the carbon tetrachloride solution was washed three times in a separatory funnel. The iodine content was determined by shaking the carbon tetrachloride solution with a standard solution of 0.016N sodium thiosulfate. The aqueous layer, which contained the inert iodine and radio-iodine as I^-, was separated from the carbon tetrachloride and boiled to expel any traces of the latter substance. The radio-iodine solution was then made up to a standard volume of 100 cc.

Method of administration and collection of samples. An exactly known volume of the radio-iodine to be given to a patient or normal subject was measured out from the standard solution, which had been prepared previously. The radioactivity of the radio-iodine administered was equivalent to from 10 to 50 μgm. of radium element in equilibrium with its decay products, using the ionization of the beta-rays from these two substances as a basis of comparison. A Lauritsen quartz fiber electroscope was used for these comparative determinations. Sufficient inert iodine in the form of sodium iodide was added to the radio-iodine solution to bring its total iodine content to 14 mgm. before it was administered. The solution was given by mouth to the subject in the morning before breakfast, and food was withheld for an hour afterwards. This interval of time was considered to be adequate since it had been found from previous studies (4) that the absorption of iodine by the way of the digestive tract was apparently over 80 per cent completed at the end of one hour.

The urine was collected in 24-hour samples and the volume of each sample accurately measured during a five-day period following the administration of the radio-iodine. The feces were collected in a large glass jar of 2000 cc. capacity during the five-day interval. At the conclusion

of the experiment, 1000 cc. of water were added and the mixture shaken vigorously so as to produce a homogeneous suspension of the feces. The total volume was determined.

The thyroid glands of all the patients upon whom thyroidectomy was necessary, were extirpated from 48 to 53 hours after the administration of the radio-iodine. The amount of thyroid tissue left in the patient was estimated carefully, and in most instances was not found to exceed 3 grams. This quantity was always only a small fraction of the portion removed and therefore this estimation did not introduce any significant error into the value for the total weight of the gland. The portion removed was then weighed before and after a small fraction of it had been cut for histological examination.

The control subjects consisted of a group of hospital patients who did not have thyroid disease, heart disease, or congestive heart failure, and who were afebrile during the period of the experiments. The renal function of each control subject and thyroid patient was determined by the intravenous phenolsulphonephthalein excretion test as described by Chapman (5). The excretion of the dye was found to be normal in all of the control group and in all except one of the thyroid patients. As was to be expected, the rate of excretion of the iodine in the patient with renal damage was retarded, and for this reason the data on excretion for this patient are omitted. Medication with iodine was terminated 12 to 24 hours prior to the administration of the radio-iodine, and was withheld during the 5-day period in the thyroid patients who had received pre-operative iodine medication. The control group and the rest of the thyroid patients received no iodine at any time except the radio-iodine solution. All of the patients in each group received the routine hospital diet. The iodine content of this was not determined; but it can be assumed safely that it was not more than a few tenths of a milligram.

Method of determination of the radioactivity of the samples. For three reasons the accurate determination of the radio-iodine content of the excreta and of the thyroid glands presented the most difficult and tedious phase of this work. First, the preparation of radio-iodine samples of sufficient activity involves prolonged bombardment of the tellurium by the cyclotron; second, the gamma-ray emission of the 8-day radio-iodine is very weak; and third, the beta rays are so soft that 1 mm. of tissue will screen out over 90 per cent of them. The result of these limitations was to make it necessary to find a procedure which would permit the extraction of the radio-iodine quantitatively from large amounts of the biological materials. To accomplish this end, it was necessary to modify the methods described by Leipert (6), Trevorrow and Fashena (7) and Mathews, Curtis and Brode (8).

These methods involve the action of chromium trioxide in a sulfuric

acid medium to oxidize the organic material and convert all of the iodine present to iodate. After the oxidation has been completed, the excess chromium trioxide and the iodate are reduced and the iodine is determined quantitatively. However, for the purpose of this investigation, the radio-iodine had not only to be removed quantitatively from the biological materials but also to be obtained in a state suitable for the determination of its radioactivity.

The apparatus for these studies consisted of a 1000 cc. Claisen flask which was equipped with ground glass joints and a thistle tube. The delivery tube was bent to a right angle. The reagents required were concentrated sulfuric acid; aqueous solution of chromium trioxide dissolved in equal weight of water; oxalic acid; carbon tetrachloride; and a standardized solution of 0.008 N sodium iodine and 0.016 N sodium thiosulfate. The thiosulfate solution was made alkaline by the addition of 0.400 gram of anhydrous sodium carbonate per liter of solution before it was standardized. An analytical grade of these chemicals was considered to be satisfactory, and no purification was necessary since the iodine impurities would be too small to affect the final iodine analyses.

PROCEDURE. *Urine.* A sample of urine, from 50 to 250 cc. depending upon the radioactive strength of the sample, was added to the flask, together with 5 cc. of the 0.008 N sodium iodide. The purpose of the iodide was to act as a carrier for the radio-iodine and to permit a check of the completeness of the extraction. Eight cubic centimeters of the chromium trioxide solution were added for each 50 cc. of urine used. Fifty cubic centimeters of sulfuric acid were added slowly to the mixture when the volume of chromium trioxide employed did not exceed 20 cc. When from 20 to 40 cc. of this chemical were required, then 100 cc. of sulfuric acid were used. The mixture was distilled in a hood until the excess chromium trioxide precipitated out, and then 30 cc. of water were added cautiously and the distillation continued for an additional 5 to 10 minutes. The purpose of the prolonged distillation was to ensure complete oxidation as well as to expel most of the chlorine present in the sample which would otherwise have obstructed seriously the subsequent removal of the iodine. At the conclusion of the second phase of the distillation, 50 cc. of water were added by allowing it to suck back through the delivery tube in order to wash it free from any traces of chlorine. After the flask had cooled to about 50°C., from 10 to 50 grams of oxalic acid were added cautiously by removing the thistle tube and dropping the crystals into the mixture. Care had to be taken that the contents of the flask were not too warm and that the oxalic acid was not added too rapidly; otherwise the violence of the reaction would have resulted in the boiling over of the mixture. The exact amount of oxalic acid needed varied, depending upon the quantity of chromium trioxide used and also on the reducing capacity of the sample of the urine.

A great excess of oxalic acid had to be avoided since it would have resulted in the presence of reducing substances in the subsequent distillation and thereby have prevented complete recovery of the iodine. The proper amount of oxalic acid necessary to destroy the excess chromium trioxide was determined easily by observing the color of the contents of the flask as it changed from yellow or orange to deep green. When the reduction was completed, 60 cc. of carbon tetrachloride were measured out into a 150 cc. Erlenmeyer flask which was placed under the stem of the delivery tube so that the tip was well below the surface of the carbon tetrachloride. A condenser was not used and therefore the receiving flask was immersed in a bath of ice-water. A volume of sulfuric acid, equal in quantity to the acid used with chromium trioxide at the beginning of the oxidation, was added rapidly through the thistle tube into the reaction mixture. The flask was warmed until it started to boil and then the flame turned down so as to prevent the released iodine vapor from being expelled too rapidly into the carbon tetrachloride. The iodine collects in the form of tiny crystals within the delivery tube if the flask has not been heated too intensely, and is carried down into the receiver as the tube becomes warm. After the iodine crystals reached the carbon tetrachloride, the boiling of the mixture had to be continued slowly for an additional 5 minutes to drive over any remaining traces of iodine vapor. The carbon tetrachloride was then transferred to a small separatory funnel, to which had been added previously 30 cc. of water, and the mixture was shaken vigorously for a few seconds. After the two fractions had separated completely, the carbon tetrachloride was drawn off and the aqueous fraction returned to the Claisen flask. The washing was repeated with an additional 30 cc. of water which also was poured into the flask. The iodine content of the carbon tetrachloride was determined by adding small portions of the 0.016 N thiosulfate and shaking the mixture. The end point of the titration was reached when the pink iodine color disappeared. This determination was accurate within 5 per cent and therefore furnished a check upon the efficiency of each extraction. The water, which was used to wash the carbon tetrachloride, usually contained from 1 to 3 per cent of the iodine that was distilled over. A second 5 cc. of 0.008 N sodium iodide were added as carrier, and the iodine was distilled over as in the first extraction using 60 cc. more carbon tetrachloride. It was possible to use sodium iodide because the concentration of sulfuric acid was sufficiently high to oxidize the iodide to iodine. Furthermore, the use of iodide instead of iodate gave a check upon the recovery of any traces of iodide that might have been present in the aqueous fraction at the conclusion of the first distillation. The carbon tetrachloride was removed at the end of the second distillation, washed twice with water, poured into the first carbon tetrachloride fraction; and the iodine was titrated as previously. When the end point of the second iodine

titration had been reached, 0.5 cc. additional thiosulfate was added and the mixture allowed to stand for a few minutes before the two fractions were separated. The aqueous fraction, which contained all of the radio-iodine and inert iodine as iodide, was separated from the carbon tetrachloride and poured into a flat ashing-crucible 4 cm. in diameter and of 13 cc. capacity. The carbon tetrachloride fraction was then returned to the separatory funnel and washed with 5 cc. of water. After the two fractions had separated, the carbon tetrachloride was drawn off and the water poured into the crucible. The separatory funnel was then washed out with 2 cc. of water which also were added to the contents of the crucible. The solution was evaporated until completely dry, upon an electric hot plate which was operated at a temperature of from 90° to 100°C. When this was completed, the crucible was placed in a desiccator until the radioactivity of the iodine was to be measured.

Feces. The procedure for the extraction of the radio-iodine from this material was similar to that employed for the urine with three minor exceptions. First, 50 cc. of chromium trioxide were required for the treatment of 50 cc. of the aqueous suspension of fecal matter; second, 100 cc. of sulfuric acid diluted with 100 cc. of water were then added cautiously to the mixture; third, 100 cc. of sulfuric acid were added after the mixture had been reduced by the oxalic acid. Care had to be taken not to add the dilute sulfuric acid too rapidly during the oxidation since the reaction tended to be quite violent because of the large amount of organic material present.

Thyroid. The thyroid tissue was treated in a similar manner with a few minor variations. The presence of relatively large quantities of iodine in this material made unnecessary the addition of iodide to act as a carrier. From 10 to 100 grams of thyroid tissue could be treated by this procedure. Larger glands were either divided into two portions and each fraction treated separately or a larger Claisen flask employed. Two cubic centimeters of chromium trioxide solution were required for each gram of tissue. A minimum of 50 cc. of sulfuric acid diluted with 50 cc. of water was needed. When from 20 to 50 cc. of chromium trioxide solution were used, 100 cc. each of sulfuric acid and water sufficed. For every additional 25 cc. of chromium trioxide solution needed, an extra 15 cc. each of acid and water were required. The quantity of acid required after the oxidation and the reduction of the excess chromium trioxide was equal to the amount added at the beginning of the oxidation. The distillation of the iodine was carried out using the procedure that had been described previously. When the iodine recovered in the first carbon tetrachloride fraction was below 20 mgm., the usual procedure was used; but when more than 20 mgm. of iodine was obtained, the water used to wash the carbon tetrachloride was returned to the Claisen flask without the addition of the sodium iodide

solution. The traces of iodine contained in the water were recovered by distillation in fresh carbon tetrachloride. The iodine was then removed, washed twice, combined with the first fraction, and the iodine content determined. The water from the second carbon tetrachloride fraction was transferred to the Claisen flask with 5 cc. of the 0.008N sodium iodide solution and the iodine was recovered. This portion was washed twice, combined with the two other carbon tetrachloride fractions, and titrated. Frequently the total volume of the aqueous fraction after the final titration exceeded the capacity of the crucible. Under such circumstances, the crucible was filled and after its contents had evaporated it was refilled from the remaining solution and from water obtained from the subsequent washing of the carbon tetrachloride and the separatory funnel.

The stated amounts of chromium trioxide were usually adequate for the extractions, but occasionally larger quantities were required since the reducing capacity of biological materials is quite variable. Since this generally cannot be foreseen, it sometimes became necessary to add more chromium trioxide and acid during the oxidation. The color of the reaction mixture during the oxidation indicated when more of the oxidizing agent was needed. Whenever the mixture turned olive, more chromium trioxide and acid had to be added.

The efficiency of this method for all three types of biological material has been checked by the addition of known amounts of radio-iodine to non-radioactive samples and by the measurement of the radio-iodine after the recovery of the iodine. The quantity of radio-iodine recovered from urine, feces and thyroid glands ranged from 96.7 to 98.4 per cent in all of the experiments.

Measurement of the radio-iodine. A vacuum tube electrometer of the design described by DuBridge (9) was employed for measurement of the radio-iodine content of the samples. This instrument is considerably more sensitive than an electroscope, and is more satisfactory than the Geiger-Müller counter when soft beta-rays are to be measured. This type of electrometer is designed for the purpose of determining quantitatively the electrical current produced by weak radioactive sources in a large ionization chamber. With this instrument, it is possible to measure within a 5 per cent error the radioactivity of a sample whose beta-ray activity is equivalent to 5×10^{-9} grams of radium.

The ionization chamber used for the measurement of the radio-iodine samples was constructed of aluminum and had a capacity of 1000 cc. The window through which the beta-rays entered the interior of the chamber was 6 cm. in diameter and covered with an aluminum foil of about 0.00003 inch in thickness. The chamber was filled with air at the pressure of the surrounding atmosphere. The crucibles containing the radio-iodine were placed directly over the window, 5 mm. above the aluminum foil.

Before and after the series of measurements of each sample were made, the sensitivity of the instrument was checked against a known standard. Each sample and the standards were measured from three to six times. The values for the rates of excretion and the uptake of radio-iodine into the thyroid gland were determined by the comparison of the radioactivity of the samples in the crucibles with the activity of an aliquot fraction of the radio-iodine given to the patient or to the control subject.

Since the beta-rays from the 8-day radio-iodine are quite soft, it was necessary to correct for the absorption of this radiation in the contents of the crucibles. The correction factor was determined by the measurement of the radioactivity of a large series of crucibles which contained from 0.1 to 50 mgm. cm^{-2} of a mixture of sodium iodide and sodium thiosulfate and a known quantity of radio-iodine. From these experiments it was found that 1 per cent of the beta-rays was absorbed by each 0.5 mgm. of the salts in the crucibles. This made necessary an appreciable correction for the samples since the crucible containing the urine and feces extractions contained approximately 3.00 mgm. cm^{-2}, while in the case of the thyroid gland extractions the solid material in the crucibles ranged from 2.50 mgm. cm^{-2} to 10 mgm. cm^{-2}.

No corrections for the decay of the samples or standards were necessary since they both were always measured within a period of an hour.

Recently a new method has been devised for the direct measurement of the uptake of radio-iodine in the thyroid before the gland has been removed. This is accomplished by the measurement of the gamma-rays from the radio-iodine atoms taken up in the gland. In using this method, the radiation was determined with the aid of a Geiger-Müller counter (4). The radio-iodine was given to the patient as in the previous studies, but the gland was not extirpated until 5 days after administration of the radio-iodine. The radioactivity of the gland was measured at 2.0, 6.2, 25.8, 50.4, 73.0, and 120.8 hours after the radio-iodine had been given. The gland was removed 3 hours after the last measurement and the radio-iodine and total iodine content of the gland determined by the same method as was employed in the other experiments. The data obtained on the rate of uptake and discharge of the radio-iodine from the gland are given in figure 2. Unfortunately, during the first day after administration of the radio-iodine, the radiation from the rest of the body was so great as to introduce a large error in the estimation of the radioactivity of the gland. Because of the rapid excretion of the radio-iodine, the radiation from the body of the patient was negligible during the last four days of the experiment, and the error was estimated to be less than 10 per cent. The counter was checked with a radium standard before and after each determination, and the radioactivity of the gland was compared with an aliquot fraction of the radio-iodine given to the patient.

RESULTS. The urinary excretion of iodine was found to be similar in normal subjects, in patients with non-toxic diffuse or non-toxic nodular goiters, and in patients with toxic diffuse and toxic nodular goiters. In the patients with thyroid diseases, the previous administration of iodine did not alter significantly the urinary excretion of the radio-active iodine mixture. It should be remembered that in all patients who had been given iodine before the radio-active studies were begun, the administration of all other iodine was stopped 12 to 24 hours before the experimental period. The urinary and fecal excretion of iodine in all subjects is shown in tables 1 and 2 and in figure 1 (in which the ordinate represents the logarithm of the per cent of iodine excreted in the urine per day and the abscissa represents

TABLE 1

Radioactive iodine studies in normal subjects and in patients with spontaneous myxedema

Radioactive iodine given orally. Excretion in urine and feces during first five days after administration (total iodine given was 14 mgm. as a single dose).

	NORMAL SUBJECTS						SPONTANEOUS MYXEDEMA	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Urine 1st day	81.0	65.0	75.2	53.1	72.0	52.9	49.8	43.4
Urine 2nd day	5.94	10.85	12.0	19.6	13.0	17.4	26.2	33.4
Urine 3rd day	0.53	1.96	1.18	3.00	0.53	2.36	9.36	9.50
Urine 4th day	0.18	0.63	0.05	0.24	0.06	0.79	4.21	2.60
Urine 5th day	0.48	0.26	0.07	0.30	0.01	0.40	1.84	0.20*
5-day feces	0.98	2.82	0.29	1.62	0.48	0.42	2.63	2.03
Total	89.11	81.52	88.79	77.86	86.08	74.27	94.04	91.13

* Patient had a severe gastro-intestinal upset during the fifth day which was associated with a marked oliguria.

the time in days). We found a greater renal excretion of iodine in the first 24 hours than did Osborne (13). However, Osborne's results may have been influenced by the use of massive doses of iodides.

The two patients with spontaneous myxedema had a much slower renal excretion of the radio-active iodine than did any of the other patients. This retarded excretion is compatible with the physiological changes known to occur in myxedema.

The amount of iodine excreted in the feces over the five-day period was very small, ranging from 0.06 to 2.82 per cent of the radio-active mixture given, except in one case. The constipated patients who passed the dryest fecal material also excreted the least iodine through the bowel. One subject excreted 11.25 per cent in the feces, a finding which cannot be ade-

quately explained (two enemas and contamination of the fecal specimens with urine could not account for the difference and the patient had not been troubled with diarrhea).

The iodine uptake, the total weights, and the total iodine content of each thyroid gland are given in table 3. There was no consistent difference between the radio-active iodine content of the glands from patients with

TABLE 2

Radioactive iodine studies in patients with various types of thyroid disease

Per cent of orally administered radioactive iodine excreted in urine and feces over a five-day period, and of radioactive iodine taken up by thyroid in 48 hours (total iodine given was 14 mgm. as a single dose).

	TOXIC NODULAR GOITER, WITHOUT LUGOL'S	NON-TOXIC NODULAR GOITER, WITHOUT LUGOL'S		TOXIC DIFFUSE GOITER, LUGOLIZED			TOXIC NODULAR GOITER, LUGOLIZED	NON-TOXIC DIFFUSE GOITER, INVOLTED (PATIENT ALSO HAD CAROTID SINUS SYNDROME)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Urine 1st day	52.7	38.3	74.2	64.7	67.7	50.3	73.9	64.3
Urine 2nd day	29.3	11.6	3.90	10.3	19.6	20.0	12.5	3.42
Urine 3rd day	2.40	1.16	0.94	1.67	0.67		1.18	0.22
Urine 4th day	0.02*	0.21	0.18	0.37	0.26		0.09	0.20
Urine 5th day	0.17	0.20	0.17	0.26	0.08		0.03	0.18
5-day feces	1.36	0.08	0.16	2.09	0.06	0.15†	0.38	11.25
Thyroid	5.50	17.5	12.5	1.07	7.50	7.12	4.32	0.07
Total	91.45	69.05	92.05	80.46	95.87	77.47	92.40	79.64
Total thyroid iodine (mgm.)	5.08	3.06	20.8		57.3	82.5	62.3	
Weight of thyroid (grams)	69.4	54.85	87.4	23.2	39.7	71.4	81.1	10.2

* Part of sample lost.

† 2-day feces.

toxic diffuse goiters and that of the glands from patients with toxic nodular goiters; nor did the previous administration of iodine alter the uptake. One carcinomatous thyroid had a small total as well as small radio-active iodine content, as might have been foreseen. One normal thyroid, removed from a patient who had previously received iodine and had a carotid sinus syndrome, contained the least radio-active iodine of all. The thyroids from the two patients with non-toxic nodular goiters, both glands showing hyper-

plasia histologically, contained the largest amount of radio-active iodine although the total iodine of each was small.

In one experiment (fig. 2) the radio-iodine content of the thyroid gland was measured for a period of five days before its removal by means of a Geiger-Müller counter. The patient had a toxic diffuse goiter and had

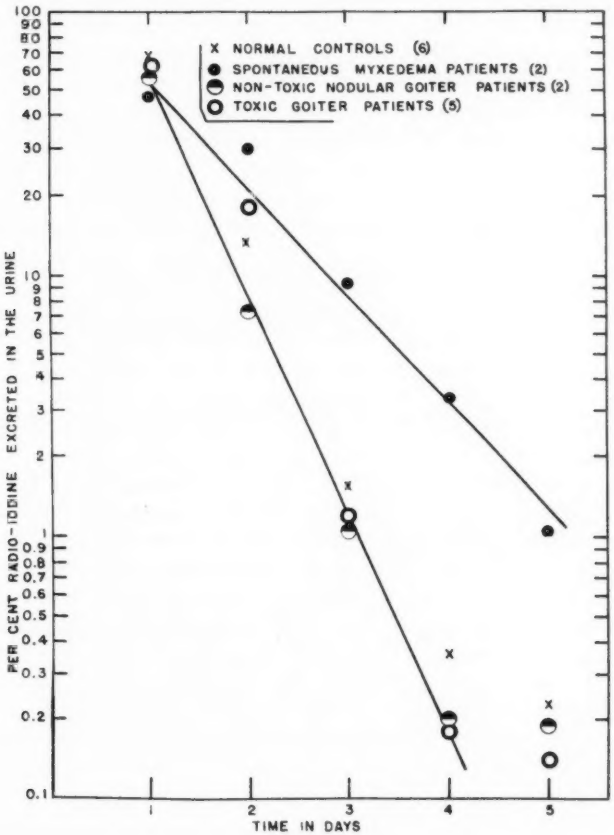


Fig. 1

received iodine prior to the administration of the radio-iodine. During the first day of the study, the measurements of the thyroid were somewhat inaccurate due to the background of radioactivity from the rest of the patient's body. The source of error was found to be less than 10 per cent during the second day and was negligible during the remaining three days of the experiment due to the rapid excretion of radio-iodine from the body.

TABLE 3

Iodine taken up by thyroid glands in various types of goiters

(Glands removed 48 hours after radioactive iodine was administered as a single dose of 14 mgm. of iodine in the form of NaI.)

	TOXIC DIFFUSE GOITER, LUGOLIZED				TOXIC NODULAR GOITER, LUGOLIZED					TOXIC DIFFUSE GOITER, WITHOUT LUGOL'S	TOXIC NODULAR GOITER, WITHOUT LUGOL'S	NON-TOXIC NODULAR GOITER, WITHOUT LUGOL'S (BOTH SHOWED HYPERPLASIA ON PATHOLOGICAL SECTION)		ADENOCARCINOMA OF THYROID	NORMAL
Total weight of thyroid (grams)	23.2	71.4	39.7	26.5	74.5	35.4	37.1	120.2	81.1	34.1	69.4	54.85	87.4	204.1	10.2
Total iodine in thyroid (mgm.)		82.5	57.3	13.9	39.6	42.8	17.85	48.9	62.3	67.	5.08	3.06	20.8	0.86	
Radioactive iodine in thyroid (per cent)	1.07	7.12	7.5	1.2	9.12	1.17	4.52	4.21	4.32	4.08	5.5	17.5	12.5	0.39	0.07

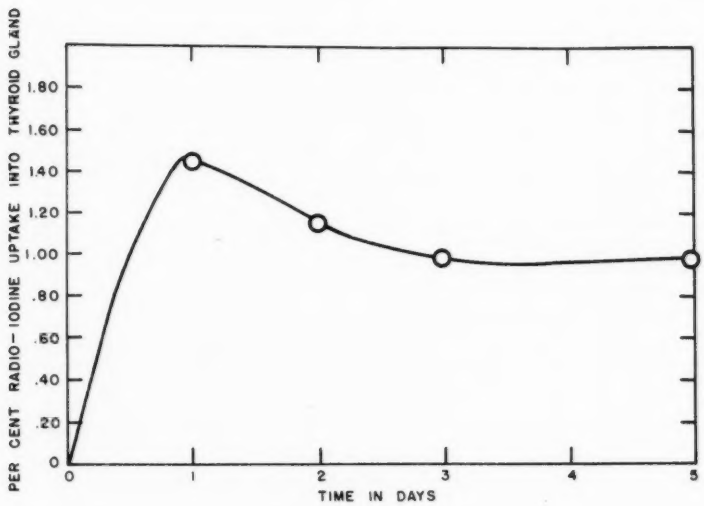


Fig. 2

Discussion. In spite of the excellent work of Marine and Rogoff (11, 12), certain phases of thyroid physiology and iodine metabolism have been impossible to study until the recent availability of radioactive isotopes of iodine with long half-lives. Iodine is a normal constituent of the thyroid gland and is present also in smaller amounts in the rest of the body

and in the excreta. Prior to the discovery of the radioactive isotopes of iodine and their application to the study of iodine metabolism, there was no possible method of differentiating the iodine given during an experimental study from the iodine already present in the body. The use of "tagged" iodine atoms solves this problem. It must be kept in mind that the non-radioactive iodine and the accompanying radio-iodine remain in constant proportion in their passage throughout the body. The weight of iodine and the content of radio-iodine of each sample are determined before the solutions are given to the patients and control subjects. Thus, determination of the percentage of radio-iodine taken up by the thyroid or excreted gives the percentage of non-radioactive iodine present in the thyroid or excreta. For example: if a thyroid gland is found to contain 5 per cent of the radio-iodine administered, then it may be concluded that the gland also took up 5 per cent, or 0.7 mgm. of the 14 mgm. of iodine given to the patient.

Most of the technical difficulties inherent in a new procedure have been overcome. However, there are some limitations which must be recognized. First, in the extractions of the radio-iodine from the biological materials there is a loss of from 1.5 to 3.5 per cent of the radio-iodine. Second, the measurements of the radio-iodine content of the samples are subject to errors varying from 1 to 5 per cent and depending upon the amount of radio-iodine present, the errors being greater for the weaker radio-active samples. Third, the small amounts of iodine lost in the sweat are difficult to recover quantitatively. Fourth, although the thyroid contains many times the amount of iodine per gram than does any other tissue of the body, still it contains only approximately 20 per cent since the muscles contain about 80 per cent of the total iodine of the body. The only accurate method of determination of the iodine in muscles is by biopsy. Since relatively large amounts of muscle tissue would have been required, none of the group was subjected to this procedure. The first two factors mentioned above are of no great significance since the errors involved are small and well within the individual variations obtained in the normal controls or even in patients having the same type of thyroid disease. The last two factors probably account in part for the variations observed in the determinations of the total radio-iodine recovered from the patients during the five-day period.

The rate of urinary excretion in the control subjects is quite constant (fig. 2). The rate of urinary excretion in the two myxedematous patients was much slower, which is to be expected in view of the general metabolic changes that occur in individuals suffering from this disease. The excretion rates in the patients with toxic goiters of either the diffuse or nodular type do not differ significantly from those observed in the control group. These findings in the patients with toxic goiters can best be explained by

the fact that they had been treated previously with iodine in amounts well in excess of physiological requirements, which resulted in saturation of the thyroid gland with iodine, and consequently in a clinical remission.

The iodine uptake of the thyroid glands in the patients with toxic goiters showed no consistent or significant relationship either to the type of toxic goiter or to the administration of iodine prior to the experimental study. This may be explained by the partial remission of the hyperthyroidism during the experiments. Had it been possible to study the more severe cases of thyrotoxicosis before the therapeutic administration of iodine, greater variations between the different groups probably would have been found. The radio-iodine uptake in the thyroid glands of the two patients with hyperplastic non-toxic nodular goiters was greater than was found in any of the other groups. This difference is in all probability a reflection of a normal output of thyroxine combined with an increased demand for iodine by the thyroid because of previous iodine deficiency. The latter factor is suggested by the relatively low total iodine content and the degree of hyperplasia of the thyroids of these two patients. The strikingly low uptake of radio-iodine in the one normal gland is understandable in view of the fact that the gland was small and the patient had previously received iodine. In the only case of carcinoma of the thyroid, the malignant tissue had both a small amount of total iodine and a small uptake of iodine. However, it is of interest to note that the ratio of the radio-iodine uptake to the total iodine content in the carcinomatous gland was exceeded only in one patient with a toxic goiter who had received no iodine prior to the experimental study and by the two patients with non-toxic nodular goiters.

The results of direct study of the iodine metabolism of the thyroid gland in one patient by the means of a Geiger Müller counter is included in this report only to emphasize the usefulness of this procedure for further investigation. A comparison of the metabolism of iodine in the thyroids of patients with varying degrees of hyperthyroidism, of patients with other types of goiters, and of normal human subjects, is possible since removal of the gland is not required. Furthermore, this method permits the study of the rate of uptake and discharge of iodine by the thyroid, which will give a dynamic picture of the iodine metabolism of normal and goitrous glands. It is of interest to note that in one patient studied by this method, the loss of iodine after the first day was quite slow (fig. 2); this observation is well corroborated by the fact that clinically the patient was in an iodine-induced remission during the five-day period of the experiment.

SUMMARY

A new method for the study of iodine metabolism in normal human subjects and patients with several different types of thyroid disease has

been described. This method employs the use of a long-lived mixture of radioactive isotopes of iodine. A detailed account is given for the method of preparation of the radio-iodine, its administration, and the procedures employed for the quantitative determination of the radio-iodine content of the excreta and thyroid gland.

The series described in this report is as yet too small to justify any definite conclusions concerning the iodine metabolism in various types of thyroid disease.

APPENDIX. Considerable investigation of the possibility of the presence of nuclear isomerism in the 8-day radio-iodine and the accompanying small amounts of the 4-day and 13-day radio-iodine, was necessary before the data obtained in these metabolic studies could be assumed to be valid. The phenomenon of nuclear isomerism is present when the nuclei of a given radioactive isotope can exist in two states. Each of the two states will have a different half-life. The upper one, which possesses more energy, is said to be metastable. A nucleus is in a metastable state when it is in a highly unstable form but can exist for an appreciable period of time at the metastable level. There are two different types of isomeric decay. In the first type, each of the two isomers may decay directly by the emission of a gamma-ray. Under these circumstances, they will decay independently of each other and therefore behave as a mixture of two radioactive isotopes of the same element. Occasionally certain radio-elements possessing a pair of isomers (notably the radio-bromine of atomic weight 80) undergo a more complicated type of decay. When this second variety of disintegration occurs, the metastable nucleus, which possesses the higher energy, drops down to the lower isomer which possesses less energy. When this process takes place, a gamma-ray is emitted from the metastable nucleus. The gamma-ray gives up its energy to an electron lying in the innermost shell of the atom. This results in the ejection of the electron and is immediately followed by the emission of the characteristic x-ray of the element when the vacancy in the shell is filled by another electron. The disturbance created in an atom by this phenomenon is so great as to break any chemical bonds that the atom may have acquired previously. It must be remembered that the shift from a higher to a lower isomeric level involves only the release of energy from the nucleus and that the lower isomer is still the same element.

The first type of isomeric decay is of no consequence in tracer studies since the pair of isomers will behave as a mixture of two radioactive isotopes of the same element. The second type of nuclear isomerism would be a serious source of error in a metabolic study should the radio-element used possess this property. This error would result from the fact that radioactive atoms which had undergone chemical combination in the body, such as iodine in thyroxine, would be released from their compounds when the transition from the upper to the lower isomeric level took place. Segre and Seaborg (10) have demonstrated experimentally the chemical separation of the isomers in Br^{80}. They synthesized tertiary butyl bromide from the radio-bromine and were then able to recover more than 80 per cent of the lower isomer as it was released from the compound.

The 8-day radio-iodine with its other two associated 4 and 13-day isotopes and the 25-minute radio-iodine used in previous absorption studies (4) were examined for the possible presence of nuclear isomers undergoing the second type of decay. The isotope mixture of the 8-day period and the 25-minute period were examined for iodine x-rays and none were found. An additional check upon the 8-day group was

made by the synthesis of a radioactive ethyl iodine from the 8-day mixtures of radio-iodine isotopes, and a chemical separation was made for any isomers that might be present. This experiment also yielded negative results which further corroborated the evidence of the absence of this second type of isomerism in the 8-day group.

ACKNOWLEDGMENTS. The authors wish to express their appreciation to Prof. Ernest O. Lawrence and his co-workers in the Radiation Laboratory for the preparation of the radio-iodine used in these experiments and for their advice concerning physical measurements. The suggestions and criticisms offered by Dr. John H. Lawrence of the Department of Medicine, Prof. Edwin McMillan of the Radiation Laboratory, and Prof. Thomas D. Stewart of the Department of Chemistry, are gratefully acknowledged. The thyroid glands were obtained through the coöperation of Drs. Glenn H. Bell and Horace J. McCorkle of the Department of Surgery.

REFERENCES

(1) LIVINGOOD, J. J. AND G. T. SEABORG. Phys. Rev. **54:** 775, 1938.
(2) KAMEN, M. AND S. RUBEN. Unpublished data.
(3) HERTZ, S., A. ROBERTS AND R. D. EVANS. Proc. Soc. Exper. Biol. and Med. **38:** 510, 1938.
(4) HAMILTON, J. G. This Journal **124:** 667, 1938.
(5) CHAPMAN, E. M. AND J. A. HALSTED. Am. J. Med. Sci. **186:** 223, 1933.
(6) LEIPERT, T. Biochem. Ztschr. **261:** 436, 1933.
(7) TREVORROW, V. AND G. J. FASHENA. J. Biol. Chem. **110:** 29, 1935.
(8) MATHEWS, N. L., G. M. CURTIS AND W. R. BRODE. Ind. and Eng. Chem. **10:** 612, 1938.
(9) DUBRIDE, L. A. Rev. Sci. Inst. **4:** 532, 1933.
(10) SEGRE, E. AND G. T. SEABORG. Phys. Rev. **55:** 321, 1939.
(11) MARINE, D. AND J. M. ROGOFF. J. Pharmacol. and Exper. Therap. **8:** 439, 1916.
(12) MARINE, D. AND J. M. ROGOFF. J. Pharmacol. and Exper. Therap. **9:** 1, 1917.
(13) OSBORNE, E. D. J. A. M. A. **79:** 615, 1922.

THE EFFECT OF POSTURE UPON PERIPHERAL CIRCULATION

MARIUS NIELSEN,[1] L. P. HERRINGTON AND C.-E. A. WINSLOW

Contribution no. 26, from the John B. Pierce Laboratory of Hygiene, New Haven, Conn.

Received for publication July 19, 1939

A decrease in cardiac output in man, on changing from the reclining to the standing posture, has been reported by Lindhard (1913), McMichael (1938), Bazett, Scott and Sunderman (1938), and others. Bazett and co-workers found the effect small in subjects adapted to hot weather. In other recent research (Asmussen, Christensen and Nielsen, 1939) in which postural change has been made passively with body weight supported on the crotch piece of a tilting table, it has been shown that there is, together with a decrease in cardiac output, a compensatory reduction of blood flow in organs such as the kidneys and intestines, whose circulation has conspicuous functions other than oxygen transport. Some evidence indicates a peripheral vasoconstriction (Mayerson and Toth, 1938) in this vertical position but this is not completely confirmed (Roth, Marvin and Sheard, 1938).

It is well known that the skin circulation at environmental temperatures within the zone of evaporative regulation serves primarily the function of heat regulation rather than of oxygen transport. The object of the present study has been to determine whether or not there is a reduction of skin circulation in warm environments when the passive vertical[2] posture is assumed by subjects who are not heat-adapted.

METHOD. Observations were made in a constant temperature room at temperature levels of 30°[3] and 35.6°C. Postural changes were passively made by use of a steel frame table supported at the midpoint of its long dimension in a manner permitting any degree of tilting between the horizontal and the vertical. Support for the body was provided by padded cross members placed under the head, shoulders, buttocks, calves and feet. In tilted positions the crotch rested on a padded support which permitted the removal of the body weight from the feet in varying degrees. The tilting table was, in later experiments, securely bolted to the platform of a special 150 kilo scale of 2 grams' sensitivity.

[1] Rockefeller Foundation Fellow, Universitetets Zoofysiologiske Laboratorium, Copenhagen.

[2] "Vertical" here and later refers to the tilted position with feet down.

[3] Relative humidity was low and in the range from 20 to 35 per cent.

The following observations were made: rectal temperature (by means of rectal thermocouple inserted 10 to 12 cm. into the rectum), mean skin temperature (thermopile measurement at 15 body points weighted according to segmental surface areas) (Winslow, Herrington and Gagge, 1936), oxygen consumption (Benedict-Roth apparatus), evaporative weight loss, pulse rate, and blood pressure.

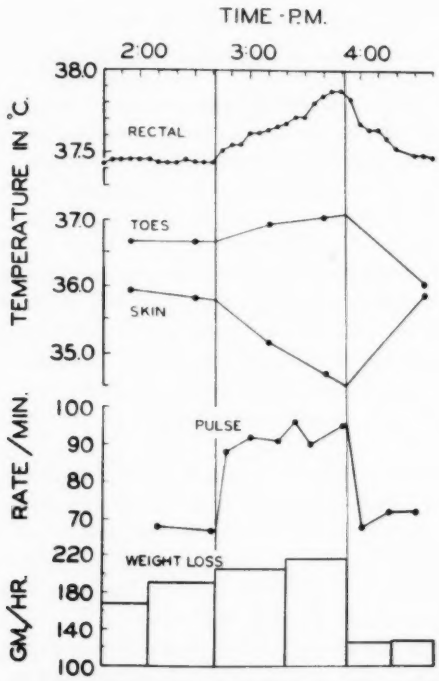


Fig. 1. Reactions to passive postural change at a room temperature of 35.6°C. First horizontal period 1:40 to 2:41, subject tilted to 45° vertical position from 2:41 to 3:52, return to horizontal position 3:52 to 4:40. *Toes* refers to toe skin temperature, *skin* to mean temperature of 15 skin points.

Three male subjects were used, but the major work was restricted to a single experimental subject (S. D.), 21 years of age, height 1.88 m., weight 79 kgm. Subjects were allowed a light lunch between 11 and 11:30 a.m., and were pre-adapted to the test environment in a reclining position from 12 to 1:30 p.m. Subjects wore only an athletic supporter. At 1:30 p.m. the subject was placed on the tilting table in the horizontal position and remained quietly for one hour. After that period the table was tilted 45° from the horizontal (feet down) for a second hour, after

which the subject was returned to the horizontal position for a final period of 40 minutes to one hour. The complete routine of measurements was usually completed at least once in each of the periods. Skin and rectal temperatures and pulse rate were taken more frequently.

RESULTS. The conspicuous facts are easily seen in figure 1, insofar as a room temperature of 35.6°C. is concerned. During the initial horizontal period the subject (S. D.) is in heat balance with the environment (35.6°) and both mean skin and rectal temperatures are essentially constant. Immediately following the change to the passive 45° posture, there is a rapid and progressive increase in rectal temperature and a decrease in mean skin temperature, which continues through the tilted hour and returns toward original values when the horizontal position is resumed in the third period. The effect is greater for the condition at 35.6° than at 30° (see table 1). We have also noted that the effect, particularly on skin

TABLE 1

Physiological measurements in horizontal and passive "vertical" postures

T_A	m T_S			m T_B			M		PULSE			S. BLOOD PRESSURE	
	Horiz.	60 min. at 45°	Decrease after 60 min. at 45°	Horiz.	60 min. at 45°	Increase after 60 min. at 45°	Horiz.	45°	Horiz.	45°	Increase at 45°	Horiz.	45°
°C.	°C.	°C.	°C.	°C.	°C.	°C.							
30.0	35.4	34.9	0.5	37.17	37.44	0.27	94.0	95.0	62	82	20	114	115
35.6	35.9	34.9	1.0	37.22	37.61	0.39	95.0	93.5	70	95	25	118	111

T_A (air temperature), m T_S (average of mean skin temperatures), m T_B (mean of rectal temperatures), M (metabolism in kilogram-calories per hour), Pulse (beats per minute), Blood Pressure (mm. Hg).

temperature, increases with greater angles of tilting, and that the toe temperature rises instead of falls with the general skin temperature. The moderate position of 45°[4] was used routinely because, at the greater angle of 70°, the subject collapsed before the routine of measurement could be completed. In experiments terminated by collapse, the skin temperature fall has been greater than in any other circumstance, and this was true of all subjects.

In table 1 are presented the means of experiments at 30° and 35.6° for S. D., and it is to be noted that the changes described above are not dependent on changes in metabolism.

DISCUSSION. The most natural interpretation of the rise in rectal temperature and the fall in skin temperature, in association with a relatively

[4] Except when otherwise noted, the term "vertical position" indicates the 45° angle of inclination.

constant metabolism, is in terms of a peripheral vasoconstriction whose function is to shunt into the general circulation some part of the blood which would normally enter the skin circulation.

The observed changes in rectal temperature cannot be summarily attributed to change in total heat loss due to the fall in skin temperature, since the total heat loss in vertical position is not decreased, but very slightly increased. Subject S. D. as an example, maintained an average difference of 5.4° between skin and air at 30° (horizontal) with a loss by radiation and convection of 50 Calories (metabolism minus evaporation), indicating an environmental constant (K_0) for $R + C$ heat-loss of 9.1 Calories per hour per degree Centigrade difference between T_S (skin temperature) and T_A (air temperature). If we apply this factor to the mean results at 35.6°, where our large evaporation loss permits a more accurate partition, we obtain the following results for the horizontal and vertical positions at the 60-minute point in each position. M, E and $R + C$ refer respectively to metabolism, evaporation, and radiation plus convection.

TABLE 2

Partition of heat loss for experiments at an air temperature of 35.6°C.

	M	E	$R + C$	NET COOLING RATE
				kgm. cal. per hour
Horizontal	+95	−93	−3	1
45° Vertical	+94	−107	+6	7

A net cooling *rate* of 7 Calories per hour for the total man at 60 minutes represents a maximum value since this estimate is based on final T_S values for the period and the *amount* of cooling in this period must be a function of some lower average rate over the period as a whole. It therefore appears that the rectal rise is not due to any decrease in heat loss, since the increased evaporation during the vertical hour must cool the body at a rate somewhat lower than 0.1° per hour.

We attribute the observed increase in rectal temperature in the vertical position, to a change in the temperature gradient from the internal tissues to the skin surface due, most probably, to a vasoconstriction in the skin, the interior of the body being heated and the peripheral tissues cooled in this position. In the same manner the rapid decrease in rectal temperature on returning the subject to a horizontal position is attributed to a vasodilatation taking place in the skin at that time. Such shifts in rectal temperature due to sudden vasoconstriction and vasodilatation in the skin are also regularly observed in the temporary phase of the adjustment which is observed when passing from a warm to a cold room or vice versa: the

rectal temperature, in spite of increase in heat loss, rising in the cold room, and, in spite of decrease in heat loss, falling in the warm room.

It is possible, from the data in tables 1 and 2, to estimate the change in heat conductance associated with the peripheral constriction (Burton, 1934) (Winslow, Herrington and Gagge, 1937). For the horizontal condition at 35.6°, 95 Calories per hour is being conducted over a gradient of 1.3° between rectum and skin, indicating internal conductance of approximately 70 Calories per hour per degree Centigrade of gradient. After 60 minutes in the vertical position, 101 Calories are being lost over a gradient of 2.7°, indicating a new conductance of about 37 Calories per degree gradient. Since these conditions are very close to thermal equilibrium (see table 2) we can place considerable reliance on these comparative values. It is interesting to note that the lower conductance of 37 is still much larger than the value which past experiments (Winslow, Herrington and Gagge, 1937) indicate as a "basal" conductance under cold conditions for this subject. This rather large change in conductance indicates, on a relative basis, the very great change in peripheral blood flow which has occurred.

In order to be certain of the validity of the above interpretation it is necessary to know that the radiation and convection loss per degree difference between the skin and environment (environmental constant) and the evaporation loss, are not changed sufficiently by tilting to explain the observed skin temperature decrease. In order to assure this, control experiments were carried out. By giving the subject full support for the feet, a practically constant level of rectal and skin temperature was reached in the "vertical" position, (at a level intermediate between those observed in the horizontal and the partially-supported vertical position), and a calculation[5] showed that the environmental constant in the vertical position increased by about 6 per cent. This corresponds to a decrease in the net cooling rate from 7 to 6.5 Calories in the text table above, which is of no significance for our problem.

In table 3 are shown the average results from two experiments at 30° in which one of the subject's legs was completely covered by a form-fitting rubber stocking. No evaporation could occur from the covered leg in this experiment but the temperature decrease in the vertical position and the temperature increase on returning to the horizontal position were as great on the corresponding midfront thigh as on the uncovered leg. This effect can only be attributed to changes in the skin circulation.

This experiment was introduced as a further direct check on the influence of evaporation. Evaporation loss is increased in the vertical position, possibly in response to a rising internal temperature. The total heat loss, in the vertical position, however, remains approximately the same as in

[5] M-E = 49 Cal. per hour; $T_S - T_A$ = 5°C; K_O = 9.8 Cal. per °C difference.

the horizontal position as a result of the smaller radiation and convection loss associated with falling skin temperatures. The direct evidence of the rubber stocking experiment and the results of our heat partitions considered jointly, indicate conclusively that no change in total heat balance or in environmental factors affecting heat loss is responsible for the decrease in skin temperature.

The vasoconstriction in the skin in the vertical position is to be related to the effect of gravity on the circulation. In the vertical position a considerable amount of blood is accumulated in the veins below the heart, particularly in the veins of the lower extremities, with a resulting decrease in venous return and in cardiac output in spite of the increase in pulse rate. With the decrease in the cardiac output, the arterial blood pressure would, other things being equal, be lowered. Previous work (Asmussen,

TABLE 3

Temperature changes in horizontal and vertical positions at 30°C. with control of evaporation on one extremity*

LOCATION	A CHANGE IN °C. (60 MIN. AT 45°)	B CHANGE IN °C. (40 MIN. IN 2ND HORIZ.)
Midfront thigh—rubber	−0.9	+0.9
Midfront thigh—nude	−0.9	+0.8
Side calf—rubber	−0.1	+0.1
Side calf—nude	−0.2	+0.3
Midfront trunk—nude	−0.4	+0.7
Rectal	+0.28	−0.19
Oral	+0.25	−0.11

* Changes noted refer to the basal level of the first horizontal period in the two experiments (A), and to the final level in the vertical period (B).

Christensen and Nielsen, 1939) has shown that the decrease in the cardiac output is compensated by a vasoconstriction in such organs as the intestines and kidneys, where the circulation has main functions other than oxygen transport. Under these conditions, the normal arterial blood pressure is maintained but at the cost of a diminished supply of blood to these organs.

In hot environments the skin circulation is greatly increased in the service of heat regulation. The results of the present experiments show that on tilting from a horizontal to a vertical position, vasoconstriction occurs in the skin with a resulting maintenance of arterial blood pressure (table 1) in this position; or (at 35.6°) with only a slight decrease. The small decrease in the arterial systolic blood pressure and the greater increase in pulse rate on tilting at 35.6° seem to indicate that the compensation through vasoconstriction in the skin is less complete in the hot room at

35.6° than at the more moderate temperature of 30°. The vasoconstriction of the skin in the vertical position modifies the heat regulatory function, a compensation in the interest of the general circulation which occurs at the cost of a rise in the internal temperature of the body. The recent work of Bazett, Scott and Sunderman (1938) indicates that these effects might not be conspicuous in subjects whose blood volume has been increased by heat adaptation.

An increase in evaporation compensates, to a certain degree, the thermal effect of vasoconstriction. This apparently maintains heat loss at a normal value but in association with a greater difference between skin and internal temperature.

The increase in sweat secretion in the vertical position is possibly stimulated by increase in internal body temperature, and the decrease in sweat secretion below normal often seen in the second horizontal period is possibly due to the decrease in internal body temperature.

As mentioned earlier, the skin temperature of the toes—and to a smaller extent of the instep of the foot—changes on tilting in an opposite direction to the temperature of the main body surface. These areas increase in temperature on tilting to the vertical position and decrease on a return to the horizontal position. A similar increase in temperature of the toes on changing from a lying to a standing position has been reported by Roth, Marvin, and Sheard (1938). This contrary behavior of the temperature of the toes may probably indicate a protective mechanism which prevents undue cooling of the feet and toes under conditions of general peripheral vasoconstriction. As a result of the low metabolism, small heat capacity, and large cooling surface, it would seem to be of importance that the circulation in the toes, on exposure to cold, be increased instead of decreased as in the skin on the main body surface. It is conceivable that the increased blood flow through the feet in the vertical position is related in part to increased hydrostatic pressure in the blood vessels of the feet. On passive change to the vertical position the hydrostatic blood pressure changes are equal in the veins and arteries of the feet. The cross-sectional dimension of the smaller vessels may increase due to the increased pressure, so that the circulation would increase in accordance with a smaller resistance against the blood flow. Such a mechanism has been suggested by Goldschmidt and Light (1925) who, in several cases, found an increased circulation in the hand when the arm hung passively down. It is also possible that a local difference in vascular anatomy, in particular an increased number of arterio-venous anastomoses, may permit a rapid blood flow in spite of general peripheral capillary constriction. The temperature of the back of the hand and of the finger was always, in our experiments, quite high, indicating a very rapid circulation in the horizontal position, and increases in the vertical position were not regularly seen as was the case

with toe temperature. No change, or an increase, was the usual experience. It is quite interesting that in experiments at a high tilting angle (70°), at which collapse was usual, both fingers and toes decreased in temperature with the general skin surface.

CONCLUSIONS

1. A passive change of body posture on a tilting table from the horizontal to a feet-down position (45° from horizontal) produces a peripheral vasoconstriction in subjects not adapted to warm environments.

2. The degree of vasoconstriction increases with progressively warmer environments and higher angles of tilting. Higher angles of tilting or very hot environments are associated with a tendency to collapse.

3. A rise of internal body temperature, roughly proportional to the degree of vasoconstriction, occurs in the vertical position and may be functionally associated with the stimulus to increased evaporation.

4. The disturbance of the heat regulatory function due to vasoconstriction in the tilted position is, to a certain degree, compensated by an increase in evaporation.

5. Except under very severe conditions, toe temperatures change in an opposite direction to general skin temperatures, possibly involving a protective mechanism against local chilling.

REFERENCES

ASMUSSEN, E., E. H. CHRISTENSEN AND M. NIELSEN. Skand. Arch. f. Physiol. **81:** 214, 1939.

BAZETT, H. C., J. C. SCOTT AND F. W. SUNDERMAN. This Journal **123:** 11, 1938.

BURTON, A. C. J. Nutrition **7:** 497, 1934.

GOLDSCHMIDT, T. AND H. B. LIGHT. This Journal **73:** 173, 1925.

LINDHARD, J. Skand. Arch. f. Physiol. **30:** 395, 1913.

MAYERSON, H. S. AND L. A. TOTH. Proc. Soc. Exper. Biol. and Med. **38:** 879, 1938.

MCMICHAEL, J. AND E. A. JOHNSTON. Quart. J. Exper. Physiol. **27:** 55, 1937.

ROTH, G. M., M. D. W. MARVIN AND C. SHEARD. This Journal **124:** 161, 1938.

WINSLOW, C.-E. A., L. P. HERRINGTON AND A. P. GAGGE. This Journal **116:** 641, 1936.

This Journal **120:** 1, 1937

THE MEASUREMENT OF INSULIN ACTION[1]

D. R. DRURY AND PAUL O. GREELEY

From the Department of Physiology, School of Medicine, University of Southern California

Received for publication June 28, 1939

The rôle of insulin in carbohydrate metabolism cannot be considered as one process. There are at least two actions. The first of these is that which holds in check the new formation of glucose and this is best illustrated by the effect of insulin on the fasting depancreatized dog. Even when fasting these animals have a high blood sugar and excrete large amounts of glucose which result from the excessive breakdown and transformation of body protein (1). This can be prevented by a small constant injection of insulin. Houssay (2) and Greeley (3) have made measurements of this so-called basal requirement and their figures vary between 0.0051 and 0.035 unit per kilo per hour, which is the rate at which insulin must be injected intravenously to maintain the blood sugar of the fasting depancreatized dog at a normal constant level.

The other action of insulin is brought into evidence when insulin is given at a rate greater than the basal rate. Then the blood sugar will fall rapidly and glucose must be given in order to maintain the blood sugar in a steady state. This action is quite different from that controlling the new formation of glucose; it is rather a matter of removal of glucose from the blood and tissue fluids and disposal of it by oxidation or conversion to storage compounds such as glycogen and fat. The intensity of this action, or its activity, can be readily quantitated by determining the rate at which glucose must be injected to balance it and so maintain the blood sugar at a constant normal level.

It is this action of insulin, i.e., disposing of glucose, which is most in evidence after administration of ordinary doses of insulin (since the amount of insulin concerned with the basal requirement is quite small) and hereafter when we speak of "insulin activity" we have in mind this "glucose disposing" mechanism. In the past workers have attempted to quantitate this activity by determining the total amount of glucose that a given dose of insulin is able to dispose of—the so called glucose-insulin equivalent (4). We believe such a method inadequate since a given dose of insulin exerts its effect throughout a certain period and the intensity of

[1] The insulin used in this work was contributed by the Eli Lilly Company.

its action changes with time. To give the complete picture of this action then one must do so with respect to time, measuring the activity throughout the period of the action of the dose. The integration of a time-activity curve for a given dose of insulin would then give us the "glucose-insulin equivalent" but this integrated value itself does not give us the complete description of the action of the dose since there could be an infinite number of time-activity curves which could give the same integrated value. We maintain then that one should consider insulin action in a dynamic sense; at any given time after an injection of insulin there is in the body a certain insulin activity which can be definitely measured by measuring the intravenous glucose rate just necessary to balance it at that time.

METHODS. We used depancreatized dogs entirely in our work in order to eliminate the unknown amount of insulin secreted by the pancreas. After pancreatectomy the animals were carried on insulin for one month to allow for complete recovery. Thereafter they were maintained at a constant weight on insulin and a measured diet of raw pancreas, meat and sugar with adequate vitamins and a small amount of sodium chloride. A given dog was not used more than once a week for a determination.

For the test the dog received his last food and insulin 24 to 36 hours before the start of the run. Some insulin was given intravenously the previous evening. On the morning of the experiment two or more units of insulin were given intravenously. The purpose of this was to bring the blood sugar level down to normal; the amount needed was established by previous trial, and once determined was quite constant for a given animal. Blood sugar determinations were made every 30 minutes by the Folin-Wu micro method. After the blood sugar had reached a normal or subnormal level and was rising again, the test proper was begun. For the tests insulin was always given intravenously.

In our first experiment (fig. 1) we attempted to produce a condition of constant insulin activity by giving a continuous constant intravenous injection of insulin at the rate of 1.12 units (1 unit plus the basal requirement of this dog) per hour for 10 hours. The resultant activity was balanced by constant intravenous glucose, the rate of this being adjusted according to the trend of the blood sugar during the previous period of one half hour which was the time between successive blood sugar determinations. It is apparent that the activity curve increases at first and attains a maximum after 8 hours. Thereafter it becomes a plateau indicating a steady state. This stage can be most readily explained as the condition which results when the rate of removal or destruction of insulin is equal to the rate of injection of it into the blood stream.

That the blood sugar level is an indicator of the balance between the rates of glucose injection and glucose removal from the blood stream is demonstrated by the second chart which depicts an experiment similar to

the preceding excepting that after attainment of the steady state as the result of hourly injection of intravenous insulin and glucose the rate of glucose injection is varied to see if this would cause definite changes in the blood sugar level. It is quite apparent that this is the case. The average glucose injection rate is indicated by the broken line. When the injection rate is less than this the blood sugar falls and vice versa. Having thus established the adequacy of the method for the measurement of insulin

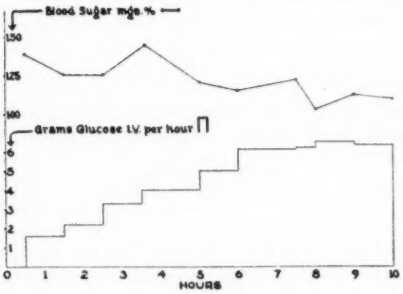


Fig. 1

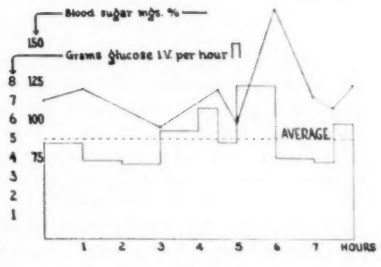


Fig. 2

Fig. 1 shows the balancing of insulin activity by constant intravenous glucose. The animal received 1.12 units insulin per hour I.V.

Fig. 2 shows the effect of increasing and diminishing the glucose injection rate on the blood sugar level after balance has been attained between insulin activity and glucose injection. The dog received constant intravenous insulin for 4 hours before the start of the chart and throughout the period shown.

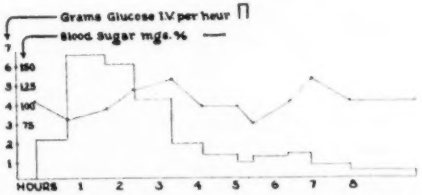


Fig. 3 shows an attempt to balance the activity resulting from a single dose of insulin (10 units) given at 0 hour.

activity, we next set about following the changing activity after a single dose of insulin. For this we usually brought the blood sugar to normal with one or two small doses, then gave the large dose to be studied and thereafter attempted to balance the insulin activity with intravenous glucose. Figure 3 shows the results of such a determination. The balance between activity and injection rate is not always exact since there are fluctuations in the blood sugar. One could readily construct the activity curve of this dose by interpolation. However, the results as they

stand really illustrate the value of the method and show that the blood sugar changes constitute a very sensitive indicator, quickly responding to small imbalances between activity and injection rate. In other words, in the blood sugar region between 80 and 160 mgm. per cent the diabetic does not have any mechanisms to "right" the blood sugar to normal: such mechanisms would interfere with the method we use. In table 1 is given the results of two runs in which balance between activity and glucose injection rate is almost exact with quite constant blood sugar levels throughout.

TABLE 1

Results of runs on two dogs in which activity of 10 units insulin is balanced by intravenous glucose. The insulin was injected at 0 hour and the blood sugar opposite this is the blood sugar at that time. In dog A no preliminary dose of insulin was given, the activity of insulin brought the blood sugar to normal and thereafter it was balanced by injected sugar. In dog B the blood sugar was first brought to normal before the injection of the 10 units. Adjustments of the glucose rate were made during the periods in accordance with blood sugar changes; blood sugar at the end of the hour is given along with the average for injection rates during the hour. During the 8th hour in dog A and the 7th in dog B the blood sugars rose although no glucose was injected showing that insulin activity had disappeared.

HOURS	DOG A		DOG B	
	Injection rate during period grams per hour	Blood sugar at end of period	Injection rate during period grams per hour	Blood sugar at end of period
0		300		125
½		210		
1		125	4.5	105
2	5.0	121	4.0	115
3	3.1	118	3.5	120
4	2.1	120	3.1	117
5	1.2	120	0.6	120
6	0.8	124	0.2	125
7	0.2	127	0	140
8	0	143		

What is the underlying basis for this type of activity curve after a dose of insulin? It is evident that the insulin is not destroyed or removed from the body at a very rapid rate (like adrenalin) but somewhat slowly since it needs some 6 to 8 hours for the activity of 10 units to disappear. The shape of the curve, with its ascent to a maximum right after injection and thereafter a steady diminution suggests that the activity at any given instant is due to the amount of insulin in the body at that time; the activity diminishes as the insulin in the body becomes less. The activity is not directly proportional to the amount of insulin in the body since insulin exerts less and less effect per unit the greater the dose. Bouckaert et al.

(5) have studied this relationship in rabbits. They have determined the amount of glucose that has to be injected to maintain the blood sugar constant after various doses of insulin. If we take their injection rate for the first hour after insulin administration we obtain a rate very near that for the activity of that amount of insulin. (The rate for the hour is, of course, the average for all rates during that hour, only that of the beginning being the rate for the amount injected; from then on the rate diminishes somewhat throughout the hour.) We have carried out similar determinations in a lower dosage range than theirs. The combined results are given in table 2, figures for doses between 0.08 and 1.3 units being ours and between 1.7 and 27.2 units being theirs. It is quite evident that the relationship of the amount of insulin in the body to the resultant activity is not a simple one. If these figures be plotted one would obtain a curve

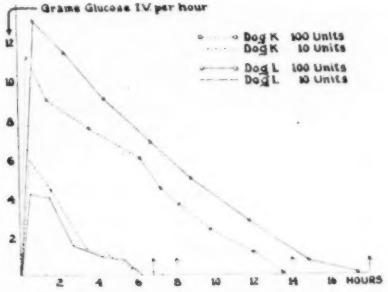


Fig. 4 shows the activity curves (the amount of glucose that has to be given per hour to keep the blood sugar constant) for 10 units and 100 units in two dogs. The arrows indicate the time when the blood sugars began to rise despite no injection of sugar.

of hyperbola type. Therefore our activity curves given above do not represent directly the curve of insulin remaining in the body, although the activity is actually due to the insulin remaining.

We have compared the effects of two different doses in the same animal. Figure 4 shows the results of such a comparison on two dogs for doses of 100 units and 10 units. These curves give additional evidence in favor of our view that the activity at any moment after the injection of a dose of insulin is due to the amount of it remaining in the body at that instant. If we consider dog K (broken line) we see that at the 6th hour after the injection of 100 units the activity is the same as immediately after the injection of 10 units, i.e., balanced by 6 grams glucose per hour. We interpret this as meaning that by 6 hours after injection the 100 units had diminished to 10 units. We notice that the behavior of the curve from this point on is very similar to that of the 10 unit curve which gives addi-

tional support to this claim. For dog L (unbroken line) a similar relationship may be observed.

Discussion. The ordinary method for measuring insulin activity has been to follow the blood sugar curve after injection, interpreting a steep drop as indicating high intensity and a flat one as low intensity. Himsworth (6) has used this method with success and has been able to demonstrate differences in insulin activity under different conditions. The method can only be considered semiquantitative however and has definite limitations, the most serious of which is that as soon as the blood sugar approaches the hypoglycemic region, mechanisms such as adrenalin secretion are brought into action which counteract the blood sugar lowering action of the insulin. It would be possible to have the blood sugar in the hypoglycemic region remaining constant or even rising at a time when considerable amounts of insulin are present in the body. Aubertin and Castagnon (7) have also used this method for the measurement of insulin

TABLE 2

INSULIN UNITS PER KGM.	GLUCOSE GRAMS PER KILO NEEDED FOR FIRST HOUR AFTER INJECTION
0.084	0.17
0.167	0.28
0.330	0.40
1.3	0.67
1.7	0.74
3.4	0.99
6.8	1.13
13.6	1.13
27.2	1.13

activity and have concluded that the same dose of insulin has a much greater activity in the diabetic than in the normal because the blood sugar fell 200 mgm. per cent in the former and 40 mgm. per cent in the latter after the same dose of insulin. With the former however the blood sugar curve started at levels like 300 mgm. per cent so that much of the glucose disappearing from the blood during the first part of the drop could have been removed by the kidneys (8). In the normal animals the blood sugar dropped to hypoglycemic levels where it was held from going lower by the mechanisms referred to above. For these reasons we believe the exact quantitative measurement of insulin activity must be carried out with the blood sugar at a normal level which can only be effected by balancing glucose utilization with intravenous injection.

There is a very definite practical value in considering the action of insulin on a time-activity basis. The present-day way of considering

insulin action—the carbohydrate equivalent per unit basis—really gives a summated effect for the entire period of action. This, however, is not adequate. The activity of a given dose of insulin should be balanced as closely as possible by the assimilation of carbohydrate from the intestine; contrariwise the carbohydrate as it is absorbed from the intestine should be balanced by the insulin activity. If absorption is not properly balanced a diabetic condition obtains. If the reverse condition results, we have insulin waste and even danger in hypoglycemia. This harmony should exist throughout the period of action. If the blood sugar rises to high levels at first due to a preponderance of glucose absorption, the harm cannot be rectified later by a hypoglycemia due to the reverse relationship. Likewise, a subsequent hyperglycemia cannot compensate for a previous low blood sugar due to unbalanced insulin activity. It is of importance, therefore, that we have an exact knowledge of both insulin activity and absorption rate. Our purpose here is to show that this method is a practicable and complete way of determining insulin activity. We have presented curves for this activity with intravenous administration. It is obvious that similar determinations should be carried out for subcutaneous administrations of different types of insulin. Having determined such curves there remains the dietetic problem of administering food in such a way that absorption from the intestine balances the insulin activity throughout its course of activity.

The common clinical method of determining insulin dosage for patients by trial and error should be modified in the light of our results. With this method the patient is put on a trial regime of diet and insulin, the 24 hour urine is collected and its glucose content determined and a certain amount of insulin is added to the dosage of the next day for each gram of sugar in the urine. We claim that attention should be given to the time of the hyperglycemia giving rise to the glycosuria, and the added insulin so given that the resultant activity will operate at the proper time to combat hyperglycemia.

SUMMARY

Insulin has two separate actions on carbohydrate metabolism in the depancreatized dog: the first or "basal" action diminishes new formation of glucose, the second produces increased removal of glucose from the blood stream to be disposed of by oxidation and storage.

The intensity of this action can be measured quantitatively by determining the rate at which glucose must be injected intravenously to balance the removal rate.

Data are presented to illustrate the manner in which the activity changes after an intravenous dose of insulin.

REFERENCES

(1) CHAMBERS, W. H. AND P. N. CORYLLOS. This Journal **78:** 270, 1926.
(2) HOUSSAY, B. A., J. T. LEWIS AND V. G. FOGLIA. Compt. Rend. Soc. Biol. **101:** 241, 1929.
(3) GREELEY, P. O. This Journal **120:** 345, 1937.
(4) ALLEN, A. N. This Journal **67:** 275, 1924.
MACLEOD, J. J. R. Carbohydrate metabolism and insulin. Longmans, Green and Co., 1926, p. 96.
(5) BOUKAERT, J. P., P. DENAYER AND R. C. KREKELS. Arch. Internat. Physiol. **31:** 180, 1929.
(6) HIMSWORTH, H. P. J. Physiol. **81:** 29, 1934.
(7) AUBERTIN, E. AND R. CASTOGNON. Compt. Rend. Soc. Biol. **118:** 697, 1935.
(8) DRURY, D. R. AND J. J. PALMER. Proc. Soc. Exper. Biol. and Med. **38:** 394, 1938.

SIGNIFICANCE OF AMINO ACIDS FOR THE MAINTENANCE OF NITROGEN BALANCE IN THE ADULT WHITE RAT[1]

PAUL A. WOLF AND RALPH C. CORLEY

From the Laboratory of Biochemistry, Department of Chemistry, Purdue University, Lafayette

Received for publication July 3, 1939

Nielsen and Corley (1939) have found that the nitrogen of mixtures of amino acids would spare completely that of the tissues of rats, fed diets low in protein. We are now reporting that, with mixtures of the same amino acids, it is possible to establish nitrogen balance in rats fed a basal diet practically devoid of protein. Requirements for growth obviously include those for maintenance. Assuming that there is no change in requirement for maintenance in the adult, and no change in the synthetic ability after adolescence, the amino acids necessary for maintenance in the mature animal must be no more numerous, and might be fewer than those essential for growth. We have been led therefore to ascertain if any of the amino acids of our mixtures, essential for growth, might be dispensable during maturity. Since the omission of each from the diet of a rat in nitrogenous equilibrium has resulted in a change to a negative nitrogen balance, while replacement has restored nitrogen balance, it would appear that the amino acids required by the growing animal are also required by the adult animal. Abderhalden has already reported (1912) that tryptophane is necessary for nitrogen balance.

EXPERIMENTAL. The general experimental procedure has already been described (Nielsen and Corley). The basal diet containing 0.01 to 0.02 per cent nitrogen had the following composition: dextrin, 30.1; sucrose, 25.2; butter fat, 36.0; cod liver oil, 3.6; bone ash, 2.9; and Cowgill's salt mixture, 2.2 per cent. The purity of each of the amino acids, prepared in this laboratory or purchased from commercial sources, has been checked by analysis for total or amino nitrogen. Optical activity has been determined for the amino acids, both isomers of which have been used. To supply water soluble vitamins, at least 100 mgm. of Harris Yeast Concentrate has been added to the mixture of amino acids for each three-day period. As it has been reported to contain vitamin B_6 (György, 1937) and as it is low in nitrogen (0.46 per cent found), blackstrap molasses, 300

[1] Based on a thesis submitted by Paul A. Wolf to the Faculty of Purdue University in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, June, 1938.

to 400 mgm. per period, has also been added. The animals acquired an unthrifty appearance after some time on experiment. This may have evidenced that the vitamin supplement was inadequate although as the animals were under study for months, even as long as six months, it seems unlikely that there was any great lack in the diet. Furthermore there is little reason to assume that even such a lack present continuously could have had any significant influence on the changes associated with the omission from and readdition to the ration of the amino acids studied.

TABLE 1

Establishment of nitrogen balance in adult rats maintained on basal "protein-free" diets, supplemented with mixtures of amino acids

PERIOD	BODY WEIGHT	BASAL DIET	URINE		FECES TOTAL NITROGEN	NITROGEN OF FOOD	NITROGEN BALANCE	AMINO ACID DIET	
			Urea and ammonia nitrogen	Total nitrogen					
	grams	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		
5	209	22	38	101	50	149	−2	40	Rat 17. 138 mgm. amino acid N
6	209	19	33	96	43	149	10	40	138 mgm. amino acid N
7	203	17	35	93	40	136	3	44	125 mgm. amino acid N
8	203	15	28	77	37	125	11	47	114 mgm. amino acid N
9	202	16	33	86	31	126	9	52	112 mgm. amino acid N
10	200	16	27	73	29	108	6	58	98 mgm. amino acid N
11	199	14	38	84	26	109	−1	58	98 mgm. amino acid N
12	197	17	35	77	27	107	3	63	96 mgm. amino acid N
4	256	19	42	107	46	157	4	39	Rat 20. 146 mgm. amino acid N
5	256	15	35	111	39	157	7	39	146 mgm. amino acid N
6	253	15	41	106	36	145	3	43	134 mgm. amino acid N
7	248	13	35	97	34	146	15	46	136 mgm. amino acid N
8	244	15	33	89	34	135	12	50	125 mgm. amino acid N
9	239	8	37	97	20	127	10	55	117 mgm. amino acid N
10	233	6	69	116	18	111	−23	61	101 mgm. amino acid N
11	231	12	44	94	13	127	20	55	117 mgm. amino acid N
12	229	14	37	85	21	117	11	66	106 mgm. amino acid N
13	223	12	41	74	11	107	22	70	97 mgm. amino acid N

Studies with the individual amino acids have been made in three ways. The amino acid has been omitted without substitute in a few cases, reducing the nitrogen intake on the experimental day. In most instances, however, the nitrogen intake on the experimental day has been kept at the control level, either by replacing the amino acid with its optical isomer or some other amino acid, or by omitting the amino acid from a mixture the nitrogen of which had been increased equivalently the preceding period by the addition of arginine. Three or more satisfactory experiments have

been conducted with each amino acid. So-called nitrogen balances do not take account of nitrogen, presumably small and relatively constant, lost by channels other than the kidney and intestine. It is understood that

TABLE 2

Milligrams of nitrogen supplied during three-day periods by individual amino acids

	AMINO ACID MIXTURES									
	43	61	106	115	120	132	139	140	143	145
l(−)-tryptophane	10	7	10	5	5	5	5	5	5	5
l(−)-histidine	12	12	12	7	7	7	7	7	7	7
l(−)-phenylalanine	12		12				12	12	12	
l(+)-isoleucine	12		12	12	12			12		12
l(−)-leucine	12	8	12	12	12	12	12	12	12	12
dl-methionine	12	10	12	12	12	12	12	12	12	12
l(+)-valine	12	12	12		12	12	12	12	12	12
l(+)-lysine	12	12	12	12	12	12	12	12		12
Synthetic threonine	40	25	40	40	40	40	40	40	40	40
l(+)-arginine			12	12						
dl-valine				15						
l(−)-cystine				6	6					
dl-phenylalanine		7		12	12	12				12
dl-alanine									12	
dl-isoleucine		8				24	24		24	
Total	134	101	146	145	130	136	136	124	136	124

104 Same as 106 except *dl*-valine for *l*(+)-valine
109 Same as 106 except *l*(−)-tryptophane omitted
113 Same as 106 except *l*(−)-phenylalanine omitted
118 Same as 106 except *d*(−)-valine for *l*(+)-valine
122 Same as 120 except *l*(+)-arginine added
131 Same as 120 except *d*(−)-isoleucine for *l*(+)-isoleucine
134 Same as 132 except *l*(−)-cystine for *dl*-methionine
136 Same as 132 except *dl*-alanine for *l*(−)-histidine
137 Same as 132 except *l*(−)-cysteine for *dl*-methionine
141 Same as 140 except 10 mgm. *d*(−)-threonine for 40 mgm. synthetic threonine
142 Same as 140 except *dl*-alanine for *l*(+)-lysine
143 Same as 139 except *dl*-alanine for *l*(+)-lysine
144 Same as 140 except 10 mgm. α-aminobutyric acid for 40 mgm. synthetic threonine
147 Same as 145 except *dl*-alanine for *l*(−)-leucine
148 Same as 145 except *d*(+)-leucine for *l*(−)-leucine

a somewhat positive nitrogen balance as calculated is required to indicate true equilibrium.

A mixture of the nine amino acids essential for growth has been found to furnish the nitrogen necessary for nitrogen balance for as long as a month, when administered to rats fed the basal ration. After this was established

a variety of mixtures of these amino acids were fed to try to ascertain the least amount of each amino acid required. This phase of the problem has not been completed. Diets 43, 132 and 141 (table 2) are representative in composition of those supplying sufficient of each of the amino acids. How much smaller amounts of each amino acid would be satisfactory we are not as yet sure.

Animals have been maintained for varying intervals in nitrogen balance with diets, the nitrogen content of which has been reduced from time to time, until the diet became inadequate. As no conclusions based on the

TABLE 3

Influence on nitrogen balance in the adult rat of the omission of tryptophane, phenylalanine or valine from the diet

PERIOD*	BODY WEIGHT	BASAL DIET	URINE		FECES TOTAL NITROGEN	NITROGEN OF FOOD	NITROGEN BALANCE	AMINO ACID DIET	
			Urea and ammonia nitrogen	Total nitrogen					
	grams	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		
43	180	19	47	124	32	160	4	104	Rat 23
44	179	16	42	113	26	145	6	43	
45	181	18	44	116	33	158	9	106	*l*(+)-arginine added
46	184	17	75	152	22	147	−27	109	*l*(−)-tryptophane omitted
47	177	9	32	101	20	144	23	43	*l*(−)-tryptophane replaced, *l*(+)-arginine omitted
48	174	15	45	115	30	156	11	106	*l*(+)-arginine added
49	178	16	65	138	23	145	−16	113	*l*(−)-phenylalanine omitted
50	175	12	39	114	22	144	8	43	*l*(−)-phenylalanine replaced, *l*(+)-arginine omitted
51	176	18	48	121	34	159	4	106	*l*(+)-arginine added
52	179	20	59	141	32	159	−14	118	*d*(−)-valine for *l*(+)-valine
53	175	15	43	122	23	157	12	106	*l*(+)-valine replaced
54	177	18	45	117	33	158	8	106	

* Periods numbered from beginning of experimental regimen; 3 days per period.

amounts of the individual amino acids in these diets are here drawn, the compositions of all but two of the diets (61 and 43) are omitted to conserve space. Table 1 presents the essential figures for observations with two rats. Amino acid mixture 61 supplying but relatively small amounts of several of the amino acids appeared inadequate for rat 20, although the low intake of food may have been important. Following a change to a different mixture of amino acids, nitrogen equilibrium has been reëstablished. While there was a marked loss of weight during the ten three-day periods, it is evident that it was associated with the loss of constituents

of the tissues other than protein, as there was in general a continuous decrease in urinary nitrogen with positive nitrogen balance.

Representative results with the nine amino acids required for growth and with arginine are recorded in tables 3 to 5. Values for fecal nitrogen

TABLE 4

Influence on nitrogen balance in the adult rat of the omission of arginine, isoleucine, dl-methionine or leucine from the diet

PERIOD*	BODY WEIGHT	BASAL DIET	URINE		FECES TOTAL NITROGEN	NITROGEN OF FOOD	NITROGEN BALANCE	AMINO ACID DIET	
			Urea and ammonia nitrogen	Total nitrogen					
	grams	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		
9	188	20	51	114	35	158	9	115	Rat 26
10	189	18	44	99	35	141	7	120	*l*(+)-arginine omitted
11	188	16	54	112	30	153	11	122	*l*(+)-arginine replaced
12	186	19	49	109	39	142	4	120	*l*(+)-arginine omitted
13	183	18	47	105	29	144	10	120	
14	183	14	38	92	29	140	19	120	
15	180	13	47	104	29	140	7	120	
16	177	12	66	137	20	141	−16	131	*d*(−)-isoleucine for *l*(+)-isoleucine
17	174	19	66	128	39	141	−26	120	*l*(+)-isoleucine replaced
18	177	20	52	114	29	147	4	132	*l*(−)-cystine omitted; *dl*-isoleucine for *d*(−)-isoleucine
19	174	17	58	117	26	146	3	132	
20	167	13	90	153	33	147	−39	134	*l*(−)-cystine for *dl*-methionine
21	165	11	106	169	24	147	−46	137	*l*(−)-cysteine for *dl*-methionine
22	163	14	83	147	31	148	−30	132	*dl*-methionine replaced
23	160	14	75	135	25	136	−24	140	*l*(+)-isoleucine for *dl*-isoleucine
24	160	14	68	126	30	136	−20	140	
25	162	16	45	101	32	136	3	140	
26	162	20	40	93	29	136	14	145	
27	161	16	52	114	34	136	−12	147	*dl*-alanine for *l*(−)-leucine
28	166	21	43	96	37	129	−4	148	*d*(+)-leucine for *l*(−)-leucine
29	165	22	44	97	48	129	−16	148	*d*(+)-leucine for *l*(−)-leucine
30	162	24	31	66	41	130	13	145	*l*(−)-leucine replaced
31	168	24	34	78	39	130	13	145	
32	165	19	30	74	33	129	22	145	

* Periods numbered from beginning of experimental regimen; 3 days per period.

indicate satisfactory absorption of the nitrogen of the diets. The effects on metabolism of omission of the individual amino acids are brought out clearly in the changes in total urinary nitrogen and in nitrogen balance. The consequence of the absence of tryptophane, phenylalanine, valine, isoleucine, methionine, leucine, histidine, lysine, or threonine respectively

has been an unmistakable *increase* in urinary nitrogen, with negative nitrogen balance. Replacement in the diet of the amino acid omitted has resulted in each instance in a *decrease* in urinary nitrogen, and a positive nitrogen balance. In a few cases (cf. periods 22–26) the recovery has been delayed, but has usually been quite prompt.

TABLE 5

Influence on nitrogen balance in the adult rat of the omission of histidine, lysine or threonine from the diet

PERIOD*	BODY WEIGHT	BASAL DIET	URINE		FECES TOTAL NITROGEN	NITROGEN OF FOOD	NITROGEN BALANCE	AMINO ACID DIET	
			Urea and ammonia nitrogen	Total nitrogen					
	grams	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		
10	186	17	45	106	31	147	10	132	Rat 27
11	186	17	38	97	36	146	13	132	
12	182	10	55	116	22	147	9	136	*dl*-alanine for *l*(−)-histidine
13	178	10	79	141	22	147	−16	136	*dl*-alanine for *l*(−)-histidine
14	172	11	59	117	32	147	−2	139	*l*(−)-histidine replaced
15	177	18	43	102	34	148	12	139	
16	176	15	44	103	31	148	14	139	
17	178	17	69	129	26	149	−6	143	*dl*-alanine for *l*(+)-lysine
18	178	16	44	103	35	148	10	139	*l*(+)-lysine replaced
19	178	15	45	105	32	148	11	139	
7	154	13	46	97	23	147	27	139	Rat 31
8	154	13	34	80	22	136	34	140	
9	155	13	43	116	34	137	−13	142	*dl*-alanine for *l*(+)-lysine
10	154	14	60	105	30	135	0	142	
11	152	13	37	84	27	136	25	140	*l*(+)-lysine replaced
12	148	13	31	65	33	106	8	141	Isolated threonine for synthetic
13	153	17	42	79	30	99	−10	144	*dl*-α-aminobutyric acid for *d*(−)-threonine. Yeast concentrate replaced by isolated vitamins
14	155	17	36	69	30	98	−1	141	
15	153	15	22	52	32	98	14	141	

* Periods numbered from beginning of experimental regimen; 3 days per period.

For the maintenance of nitrogen balance d(−)-valine did not replace l(+)-valine; d(−)-isoleucine did not replace l(+)-isoleucine; cystine or cysteine did not replace methionine; and α-aminobutyric acid did not replace threonine. The urinary values indicated that d(+)-leucine might replace l(−)-leucine, although the increased values for fecal nitrogen made the nitrogen balances negative during the time (periods 28 and

29, table 4). No final conclusions however can now be drawn as the optical activity of the preparation used was low and must have contained some (calculated 7.5 per cent) of the l-form. ($[\alpha]_D^{27°} = -15.1°$ in 20 per cent HCl; reported as $-17.3°$–$17.76°$ for d(+)-leucine.)

The omission of arginine has been followed by no significant change in nitrogen balance (periods 12–15, table 4).

Discussion. While these experiments probably indicate that the nine amino acids essential for growth can meet all the requirements of the mature rat for nitrogen, other than that provided by vitamins, it is to be noted that 8 to 14 (usually however not over 11) mgm. of nitrogen have been supplied per period in the basal ration and in the vitamin supplement, whereas 5 mgm. of nitrogen provided by tryptophane have been found sufficient per period. It is conceivable, if unlikely, that some additional amino acid, required in an amount like that of tryptophane, might be provided in the other nitrogenous constituents of the diet. Conclusive proof of the minimum requirements, qualitative and quantitative, for amino acids necessitates that the necessary vitamins be provided in pure form. With several rats, for short times (9–15 days) the yeast concentrate has been replaced per period by 0.2 mgm. of crystalline vitamin B_1, 0.06 mgm. of lactoflavin and 1 mgm. of nicotinic acid. The urinary excretion of nitrogen has remained low, but with one exception (periods 13–15, rat 31, table 5) there was such an increase in the nitrogen (and the bulk) of the feces that there was failure to establish nitrogen balance.

The omission from the complete control diets of each of the amino acids essential for growth has been followed by an increased catabolism of tissue protein as evidenced by the increase in urinary nitrogen. There was no such effect following the omission of arginine or cystine from complete diets to which these had previously been added. These observations indicate the conclusion that each of the amino acids essential for growth is also essential for the maintenance of nitrogen balance in the adult animal. It is of course possible that there are as yet unrecognized interrelationships between the amino acids. One amino acid if supplied in great relative abundance might conceivably replace one of the others, or even bring about a need for another one (cf. Gladstone, 1939).

SUMMARY

1. Nitrogen balance has been established in the adult female white rat with rations practically devoid of protein, supplemented with vitamin preparations and mixtures of the nine amino acids essential for growth, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine.

2. Each of the amino acids essential for growth appears to be required for the maintenance of nitrogen balance in the adult female white rat, as

omission of each of these amino acids from the complete control diet has been followed by a negative balance of nitrogen, while subsequent replacement has been followed by a restoration of nitrogen equilibrium.

REFERENCES

ABDERHALDEN, E. Ztschr. physiol. Chem. **77:** 22, 1912.

GLADSTONE, G. P. Brit. J. Exper. Path., April 1939, p. 189; quoted in Lancet **236:** 1165, 1939.

GYÖRGY, P. Proc. Soc. Exper. Biol. Med. **36:** 167, 1937.

NIELSEN, E. K. AND R. C. CORLEY. This Journal **126:** 223, 1939.

THE LIBERATION OF A PRESSOR HORMONE FOLLOWING STIMULATION OF THE HYPOTHALAMUS[1]

GEORGE CLARK AND S. C. WANG

From the Institute of Neurology, Northwestern University Medical School, Chicago, Ill.

Received for publication July 21, 1939

It has been shown that excitation of the hypothalamus yields both direct nervous effects and hormonal substances. Liberation of adrenin was demonstrated by Houssay and Molinelli (1925) and later Magoun, Ranson and Hetherington (1937) showed an additional liberation of circulating sympathin. The present series of experiments furnishes some presumptive evidence of the liberation of a pressor hormone from the hypophysis following electrical stimulation of the hypothalamus.

METHOD. Ten spinal cats were prepared by aseptically removing a segment of the spinal cord at the level of C_7 or C_8. Two or 3 months were usually allowed for the animals to recover their health. In 3 of these cats, the brachial plexus was severed a few days prior to stimulation.

For the acute experiments, the animal was given 15 to 20 mgm. nembutal intravenously and the right common carotid was connected to a mercury manometer for blood pressure. Stimulation was done through an insulated but bare tipped bipolar nichrome wire electrode which was connected with a Harvard inductorium (a dry cell in the primary and the secondary at 9 cm.). The stimulating electrode was introduced into the brain with the aid of the Horsley-Clarke stereotaxic instrument and the anterior part of the hypothalamus in front of the infundibulum was explored. On several occasions, artificial respiration was installed at the beginning of the experiment. The reactive region and the level of cord section were subsequently determined at autopsy.

RESULTS. In 7 of the 10 animals, in which the region dorsal to the optic chiasma, the anterior hypothalamic area, the ventromedial hypothalamic nucleus or the supraoptico-hypophyseal tract were stimulated, definite, in some cases quite marked, pressor responses were obtained. The latent period varied from 15 to 27 seconds and the rise in blood pressure continued until 105 to 220 seconds had elapsed (table 1). The rise was uniformly slow and smooth, taking generally less than half the total duration of the response to reach its peak (fig. 1). Identical responses could usually

[1] This investigation was aided by a grant from the Rockefeller Foundation.

be obtained after a 30 minute or longer rest interval, although sometimes repeated excitations were not as effective. On the other hand, in a few cases different lengths of stimulus were tried, a larger and more prolonged elevation of blood pressure usually followed the one of longer duration. In all cases there was no appreciable change in heart rate.

In 2 of these animals (cats 2 and 6) there appeared a small depressor reaction during stimulation. In the latter (cat 6), repeating the stimulation increased the fall in blood pressure which reached a maximum of 38 mm. Hg following the third stimulation of the same region. In this

TABLE 1

Maximum blood pressure variation following stimulation of hypothalamus

CAT NUMBER	INITIAL BLOOD PRESSURE	DURATION OF STIMULUS	LATENCY	DURATION OF RESPONSE	MAXIMUM BLOOD PRESSURE CHANGE	REACTIVE REGION
	mm. Hg	*seconds*	*seconds*	*seconds*	*mm.* Hg	
1	110	10	15	150	+34	Anterior hypothalamic area
2	120	15	16	150	+32	Region immediately dorsal to the optic chiasma
3	110	12	19	135	+52	Ventromedial hypothalamic nucleus
4	60	17	20	110	+32	Anterior hypothalamic area
5	90	21	24	150	+28	Region immediately dorsal to the optic chiasma
6	110	21	27	220	+60	Region of the supraoptico-hypophyseal tract
7	130	10	15	105	+31	Region immediately dorsal to the optic chiasma
	130	10	0	70	−56	Subthalamus and thalamus
8	85	9	0	45	−20	Thalamus
9	90	9	0	60	−20	Subthalamus and lateral hypothalamic nucleus
10	100	10	1	52	−24	Region dorsal to the optic chiasma, corresponding to med. forebrain bundle

cat and in another (cat 4), bilateral vagotomy was attempted and found to have no influence upon the effect of central stimulation.

Intravenous administration of 1 cc. $1/10^{-6}$ adrenaline in cat 2 resulted in a rise in blood pressure of 76 mm. Hg, lasting for $2\frac{1}{2}$ minutes; and 1 pressor unit of pitressin, a rise of 80 mm. Hg from which recovery did not take place until 8 minutes had elapsed.

In the other 3 cats (8, 9 and 10) a consistent depressor response was observed. There was no latent period, the drop was relatively sharp and the recovery comparatively rapid. They were generally reproducible as soon as the blood pressure returned to the control level. They were often asso-

ciated with vigorous neck and respiratory movements. In cat 7, marked lowering of blood pressure was obtained from the region dorsal and caudal to the pressor points; while in cat 6, as previously mentioned, the depressor response preceding the pressor amounted to 38 mm. Hg as a result

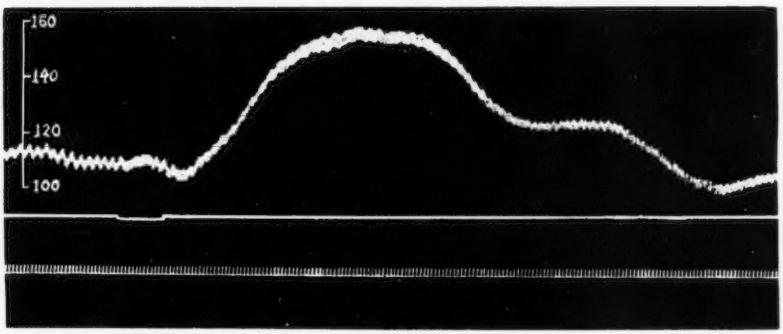


Fig. 1. Kymograph record of the pressor response. Time in seconds

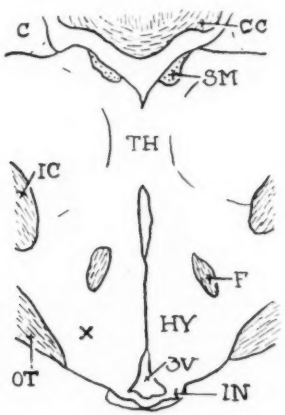


Fig. 2. Section of the hypothalamus in cat 3. The point from which the pressor response shown in figure 1 was obtained is indicated by ×. Abbreviations: *C*, caudate nucleus; *CC*, corpus callosum; *F*, fornix; *HY*, hypothalamus; *IC*, internal capsule; *IN*, infundibulum; *OT*, optic chiasma; *SM*, stria medullaris; *TH*, thalamus and *3V*, third ventricle.

of the third stimulus applied to the pressor point. Vagotomy in this cat and cat 10 did not modify this depressor response. Intravenous injection of pitressin in cat 10 yielded a pressor reaction similar to that in cat 3 although the rise was not so marked. Subsequent examination of the

loci from which depressor reactions were obtained showed most of them to be distributed more dorsally (in the subthalamus and thalamus) than the pressor points; in addition the lateral hypothalamus was found to be inactive in cat 8, while stimulation of this region in cat 9 gave a fall. The location of the depressor point in cat 10 differed in no way from the pressor points in cats 2, 5 and 7.

Comments. The long latent period and the lasting nature of the pressor response indicate that it is of hormonal origin. The fact that it is obtained after severance of the cord above $\dot{C}_5$ and vagotomy rules out the participation of sympathin and possible vagal effects. Recently, Haterius and Ferguson (1938) have reported a definite increase in uterine activity of rabbits following electrical stimulation of the infundibular region after cord section and vagotomy. They believe that the reactive agent is the liberated pitocin. It is not improbable that the pressor response reported here also represents the release of a hormone elaborated by the posterior lobe of the hypophysis, as a result of hypothalamic activation. The slow appearance of the pressor reaction could be accounted for by its gradual accumulation above a threshold level. The fact that it is not obtained exclusively from the region of the supraoptico-hypophyseal tract would indicate the existence of nervous association systems between the different hypothalamic nuclei.

Concerning the depressor reaction little need be said. Its immediate appearance with the stimulus and the fact that it may be obtained from the thalamus excludes the possibility of it being a central effect of the liberated hormone (Light and Bysshe, 1933), or the so-called inversion effect which often occurs during repeated administration of pituitary extract (Abel, 1923–24). Since this depressor effect is often associated with struggling movements, it may be similar to the depressor responses which follow struggling in the sympathectomized and vagotomized cats (Freeman and Rosenblueth, 1931; Bacq, Brouha and Heymans, 1934; Pinkston, Partington and Rosenblueth, 1936).

It would seem desirable to ascertain further as to whether this effect is due to the liberated pressor hormone from the pituitary gland by its disappearance after hypophysectomy. However, due to the uncertainty of obtaining a positive response in every animal (in this series 7 positive in 10 cases) and of repeated effects being identical, the absence of the response after extirpation of the gland might not be significant.[2]

[2] During the process of our investigation, Huang (1938) reported that a small and delayed pressor response was obtained from stimulation of the base of brain around the infundibulum in dog with isolated head preparation. It disappears after hypophysectomy.

SUMMARY

In 7 of 10 low cervical cats, stimulation of the anterior hypothalamus yielded pronounced and sustained pressor effects. The long latent period, the slow rise of the blood pressure to maximum and its persistence after vagotomy further exclude the possibility that it is of a nervous origin, or due to circulating sympathin or adrenin. An activation of the hypophysis is therefore presumed and the effect is accounted for by the liberated pressor hormone from the posterior lobe of the pituitary gland.

REFERENCES

ABEL, J. J. Harvey Lecture, p. 154, 1923–24.

BACQ, Z. M., L. BROUHA AND C. HEYMANS. Arch. internat. de pharmacodyn. et de thérap. **48:** 429, 1934.

FREEMAN, N. E. AND A. ROSENBLUETH. This Journal **98:** 454, 1931.

HATERIUS, H. O. AND J. K. W. FERGUSON. This Journal **124:** 314, 1938.

HOUSSAY, B. A. AND E. A. MOLINELLI. Compt. rend. Soc. de Biol. **93:** 1454, 1925.

HUANG, J. J. Chinese J. Physiol. **13:** 367, 1938.

LIGHT, R. U. AND S. M. BYSSHE. J. Pharmacol. and Exper. Therap. **47:** 17, 1933.

MAGOUN, H. W., S. W. RANSON AND A. HETHERINGTON. This Journal **119:** 615, 1937.

PINKSTON, J. O., P. F. PARTINGTON AND A. ROSENBLUETH. This Journal **115:** 711, 1936.

REFRACTION OF THE RABBIT'S EYE IN THE UNEXCITED AND EXCITED STATE

J. M. D. OLMSTED AND M. W. MORGAN, JR.

From the Division of Physiology, University of California Medical School, Berkeley

Received for publication July 26, 1939

The arguments in favor of a dual autonomic nervous control of the ciliary muscle, as against parasympathetic control only, have been marshalled by Cogan (1937). The theory postulates that impulses over sympathetic nerves cause radial fibers of the ciliary muscle to contract, thereby exerting a pull on the zonula and flattening the lens in accommodation for far vision; while impulses over parasympathetic nerves cause circular fibers of the ciliary muscle to contract thereby decreasing the circumference of the ciliary ring and allowing the lens to bulge in accommodation for near vision. Although Cogan's clinical observations are most suggestive, practically the only experimental evidence for the theory is that of Byrne (1933), cogent objections to which have been set forth by Cogan.

The rabbit readily shows sudden dilatation of the pupil upon being startled or excited in struggling. If accommodation for far vision should accompany dilatation of the pupil in the excited state this would afford presumptive evidence of sympathetic influence on ciliary as well as iris musculature. The rabbit's eye can be tested for near and far vision by skiascopy in the same manner as the human eye. However, the shadow can be more easily seen in dark-eyed rabbits than in albinos.

Six dark-eyed rabbits, accustomed to handling, were chosen at random and their eyes refracted under different conditions. It was found essential that for at least four minutes prior to the reading, and during the reading as well, the rabbit be gently held quiet in a comfortable position to avoid excitement. The readings were all made in dim light. During skiascopy the lowest powered convex lens that would give "against motion" was taken as the lens that represented the degree of hypermetropia existing in the rabbit's eye.

Observations from day to day under these conditions gave fairly constant readings. A typical record is shown in table 1. The lenses used were in 0.50 D steps, therefore a variation of 0.50 D from one day to the next is of little significance. In the case of the human subject, however, where coöperation can be obtained, if lenses in quarter diopter steps are used, it should be possible to have a series of readings over this same time interval with a variation of approximately 0.25 D.

In only one rabbit were the readings for both eyes identical. The aver-

age of 48 readings on normal eyes of rabbits in the unexcited state was +3.50 D for the right eye and +2.75 D for the left eye. Since the human eye in the emmetropic state is focused at 20 feet, the rabbit's eye in the resting state is much more hypermetropic than man's. A peculiar phenomenon observed during the course of measurements was the appearance at irregular intervals of astigmia greater than 0.50 D in five out of the six rabbits. This may possibly be due to oblique refraction, although care was taken to try to avoid this.

When the rabbits were startled by a sudden tap on the nose (the most convenient stimulus), by having their heads sharply raised and lowered or turned from side to side, or by being suddenly jostled about, or upon hearing a sudden noise such as the slamming of a door, they immediately became more hypermetropic at the same time that their pupil dilated.

TABLE 1

Readings in diopters

Rabbit 1

DATE	NORMAL				HOMATROPINIZED			
	Unexcited		Excited		Unexcited		Excited	
	Right eye	Left eye	Right eye	Left eye	Right eye	Left eye	Right eye	Left eye
6/ 5/39	+3.25	+1.00	+3.50	+2.50	+4.50	+3.00	+6.00	+3.00
6/ 6/39	+3.00	+1.25		+3.00	+3.50	+2.50		+3.00
6/ 9/39	+2.50	+2.00		+2.50	+3.50	+3.00		+3.50
6/14/39	+3.00*	+2.00*	+4.00		+3.50	+3.00		+3.50

* Astigmia greater than 0.50 D present.

Here an increase of 0.50 D is significant since the change was directly observable. If the stimulus was a tap on the nose or a sudden noise, the "against motion" could be seen to change to "with motion," without the observer's having to alter the position of his lens. In case of the other types of stimuli, a reading taken immediately before and again after the stimulus showed this change. The average increase (48 readings) was just under 1.00 D for the right eye, and just over 1.00 D for the left.

According to the usually accepted theory of only parasympathetic control of the ciliary muscle an increase in hypermetropia under these conditions should be impossible, since the eye in the unexcited condition should theoretically be at rest, i.e., accommodated for extreme far vision with the ciliary muscle completely relaxed.

Within 2 to 5 minutes after the stimulus, the original refractive condition had returned. Its gradual return paralleled the course of constriction of the pupil to the diameter of the unexcited state. It was observed that any astigmia which might have been present in the unexcited state sometimes decreased, sometimes increased as the rabbits were excited, though again this might be due to oblique refraction.

In order to show that this reaction was mediated by the sympathetic nervous system, the same procedure was followed after instillation of a few drops of 2 per cent homatropine into the eye to paralyze the parasympathetic endings. Within half an hour there was no response of the pupil to light and it was then found that the unexcited rabbit's eyes were even more hypermetropic than before, the average increase (48 readings) being almost exactly 0.50 D for each eye. In a study of the effect of homatropine on the human eye, Tait and Sinn (1936) found that there was no effect on ciliary tone in 70 per cent of the 150 eyes they examined, but in 21 per cent there was an increase in hypermetropia and in 9 per cent a decrease.

If the rabbits with pupils immobilized by homatropine were now suddenly startled by the methods described above, their eyes promptly became still more hypermetropic, the average increase for the right eye being nearly 1.00 D, and for the left eye, 0.50 D.

The reaction to a sudden stimulus by increase in hypermetropia in both normal and homatropinized eyes would seem to be another of the series of manifestations of adreno-sympathetic stimulation in the excited state. The results can only be explained on the assumption that the sympathetic nervous system is involved in the process whereby the lens in the startled rabbit's eye becomes even less convex than it is during the normal resting state. This strongly suggests that active accommodation for far vision in the normal eye is brought about not only by inhibition of the III nerve, but by excitation of the sympathetic nerve as well. If this is the case, it would be expected that the principle of "reciprocal innervation of antagonistic muscles" would hold here for the ciliary muscle as it does for the muscles of the iris (Gullberg, Olmsted and Wagman, 1938).

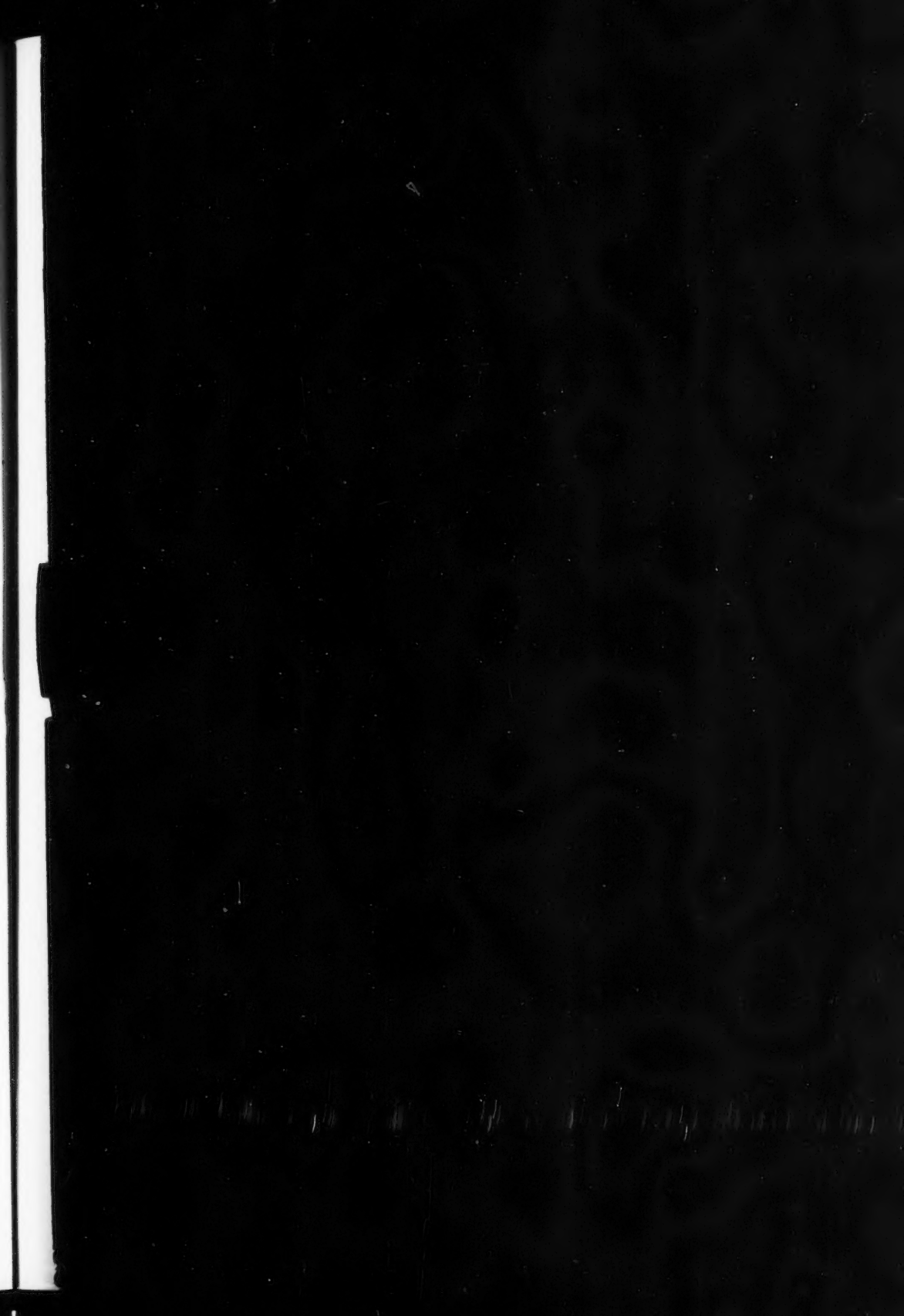
SUMMARY

The rabbit's eye in the unexcited state is more hypermetropic than man's eye at rest. Excitement causes increase in hypermetropia concurrently with dilatation of the pupil. Return of the lens to the normal resting condition parallels the course of constriction of the pupil to the diameter of the unexcited state. Homatropine increases the degree of hypermetropia over that of the normal eye, and excitement still further increases the hypermetropia. This is taken as evidence of sympathetic innervation of the ciliary muscle.

REFERENCES

Byrne, J. G. Studies on the physiology of the eye. London, H. K. Lewis and Co., Ltd., 1933.

Cogan, D. G. Arch. Ophthal. **18:** 739, 1937.

Gullberg, J. E., J. M. D. Olmsted and I. H. Wagman. This Journal **122:** 160, 1938; Proc. Soc. Exper. Biol. and Med. **38:** 616, 1938.

Tait, E. F. and F. W. Sinn. Am. J. Optometry **13:** 140, 1936.